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Description

Background of the Invention

Peptic ulcer disease exists in two forms, duodenal ulcers and gastric ulcers. Central to the cause of duodenal ulcers, is the production of excess stomach acid and pepsin and a rapid gastric emptying time. This results in an increase in duodenal exposure to secreted acid and enzymes, and in mucosal damage.

The second form of the disorder, gastric ulcer disease, may be caused by increased stomach acid and a breakdown of the complex stomach defenses that normally protect the gastric mucosa from acid damage. Although the two conditions have different etiologies, both benefit from a reduction in gastric acid secretion.

Because excess stomach acid is a central cause of ulcers, antacid preparations are commonly used as one method of treatment. This method merely neutralizes stomach acid after it is produced. Consequently, large quantities of antacids must be consumed on an ongoing basis to neutralize acid which is continually produced in the stomach. Antacids do not cure the disease because they do not affect the source of acid production.

Gastric acid is produced in a specialized stomach cell, the parietal cell. Parietal cells can be stimulated to secrete acid by acetylcholine, histamine and gastrin, upon the binding of each of these compounds with specific receptors on the surface of the cell. Of these the most potent stimulator of acid secretion is the peptide hormone gastrin.

Current approaches to the control and cure of peptic ulcers center upon devising drugs that inhibit the ability of one or more of these compounds to stimulate acid production or secretion. The most effective group of drugs approved for sale are the H2 antagonists (e.g. "Tagamet" and "Zantac") which block the histamine H2 receptors on gastric parietal cells and inhibit acid secretion. These drugs, however, require relatively large doses on a daily basis and may induce several undesirable side effects. In cases where H2 antagonists have cured ulcers, relapses occur in almost 100% of cured individuals within a year of discontinuation of treatment. Other drugs have also exhibited problems, including low efficacy and unacceptable levels of toxicity. In the case of the peptide hormone gastrin, no successful chemical antagonists have been identified.

Gastrin has several important functions in the gastrointestinal tract, the two most important being stimulation of acid secretion and stimulation of the growth of cells in the gastrointestinal tract. The hormone exists in at least two molecular forms, heptadecagastrin ("G_{17}") and tetrateseracontagastrin ("G_{34}") named according to the number of amino acid ("AA") residues in each molecule. G_{34} and G_{17} are identical in structure at the carboxy terminus, which is the binding site of the hormones with receptors. Apart from having a pyroglutamic acid as the N-terminal residue, instead of glutamine, G_{17} constitutes the 17 carboxy terminal ("C-terminal") end residues of G_{34}. G_{34} consists of the 17 C-Terminal end residues which comprise G_{17} and an additional different amino acid sequence of 17 amino terminal ("N-terminal") residues. When G_{34} is split by trypsin a G_{17} subunit and a non-hormonal 17 amino acid subunit results. Though G_{17} is usually obtained by trypsin cleavage of G_{34}, each form may also be generated separately from its own prohormone.

Although G_{17} and G_{34} are thought to be equipotent on a molar basis as stimulators of acid release, G_{17} is the primary stimulator of meal induced gastric acid secretion. G_{17} is 1500 times more potent than histamine and makes up 90% of the antral (stomach) gastrin. G_{17} accounts for roughly 60%-70% of the gastrin-mediated acid release.

The prior art in the area of gastrin immunology mainly concerns the induction of antibodies useful for identifying anatomic sites containing or producing gastrin G_{17} or G_{34} in laboratory animals; see Sugano, K., et al., 1985, "Identification and characterization of glycine-extended post translational processing intermediates of progastrin in porcine stomach", J. of Biological Chemistry 250: 11724-11729; Vaillant, C., et al., 1979, "Cellular origins of different forms of gastrin: The specific immunocytochemical localization of related peptides. J. Histochem Cytochem 27:932-935; Larsson, L.I. et al., 1977, "Characterization of antral gastrin cells with region-specific antisera". J. Histochem. Cytochem 25: 1317-1321. The antisera reported in these publications contained antibodies of numerous specificities, for a variety of antigenic epitopes on gastrin molecules.

Attempts to control gastrin levels by anti-gastrin antibodies induced by active immunization or passive administration of preformed antibodies such as those reported in Jaffe, B.M., et al., 1971, "Gastrin resistance following immunizations to the C-terminal tetrapeptide amide of gastrin, Surgery 69: 232-238; Jaffe, B.M., et al., 1970, "Inhibition of endogenous gastrin activity by antibodies to the carboxyl terminal tetrapeptide amide of gastrin", Gastroenterology 58: 151-156; Jaffe et al., 1969, "Inhibition of endogenous gastrin activity by incubation with antibodies to the C-terminal tetrapeptide of gastrin. Surgery 65: 5633-639

are different from the present invention in that the immunogen used was derived from the carboxyl terminal tetra-peptide amino acid sequence common to G_{17}, G_{34}, and to another important hormone, cholecystokinin ("CCK"). The immunogen of Jaffe et al. is thus of no practical value as an anti-gastrin vaccine component; on the contrary, it would produce a deleterious state in which all gastrin activity and other hormone function of G_{17}, G_{34}, together with CCK, would be blocked and eliminated by immunization.

Summary of the invention

This invention provides a novel immunological approach to the control and regulation of gastrin-induced disorders such as peptic ulcers, or other diseases which appear to be related to the hormonal and stimulatory effects of gastrin, and of conditions in which gastrin is over-produced. According to the invention, antibodies are induced in the patient by active immunization with immunogens that selectively target G_{17}. Such immunogens are defined in claims 1 and 9. Alternatively, the patient can be passively immunized with anti-gastrin antibodies specific for G_{17}.

A first area of major medical importance for which the neutralization of gastrin hormonal activity has great therapeutic potential concerns the control of tumors and pathological conditions that are stimulated by gastrointestinal hormones. Several cancers of the gastrointestinal tract and associated tissues are stimulated to grow by the trophic action of gastrin. See Lamers, C.S.H.S., and Jansen, J.B.M.S., 1988, "Role of Gastrin and Cholecystokinin in Tumours of the Gastrointestinal Tract", Eur. J. Cancer Clin. Oncol. 22: 267-273. Gastrin promotes the growth of colon carcinoma, gastric carcinoma and gastric carcinoids. Gastrin antagonists may inhibit the growth of human colon cancer and enhance host survival as has been shown in mice; see, Beauchamp, R.O., et. al. 1985, "Proglumide, A Gastrin Receptor Antagonist, Inhibits Growth Of Colon Cancer And Enhances Survival In Mice." Ann. Surg. 202: 303-309. The neutralization of gastrin tumor promoting activity may provide an important therapy for these diseases.

A second important application of gastrin neutralization therapy concerns conditions in which the hormone is overproduced. Certain cancers of the gastrointestinal tract, apudomas, produce extremely large quantities of gastrin. In either case, the excess hormone produced by the apudoma or pituitary tumor will have adverse physiologic effects on organs or tissues containing receptors for the hormone. Excess gastrin production by apudomas stimulates hypertrophy of the acid secreting epithelium of the stomach, leading to excess stomach acid secretion, peptic ulcer, and neoplastic changes in the epithelium.

Available treatment for tumors stimulated by gastrin and for tumors that produce gastrin consists primarily of surgical resection of the cancerous tissue. This approach is frequently unsuccessful; in many instances the tumors cannot be located or are present in anatomic sites that are inoperable. In most instances these tumors do not respond well to radiation or chemotherapy regimens. New treatments are needed to supplement present procedures.

A therapeutic method of selectively neutralizing the biological activity of these hormones would provide an effective means of controlling or preventing the pathologic changes resulting from excessive hormone production.

The method of cancer therapy described in this invention has several advantages over present treatment methods. The method is non-invasive, selectively reversible, does not damage normal tissue, does not required frequent repeated treatments, does not cross the blood brain barrier and has reduced side effects.

The therapy may be selectively reversed by injecting the patient with a pharmaceutical composition comprising a neutralizing epitope molecule. This molecule should comprise the epitope sequence free of an immunogenic carrier. This non-immunogenic molecule will bind to the free antibodies previously induced against the epitope in the host.

Brief Description of the Figures

Figure 1: illustrates the stomach acid secretions over time of a control rat injected sequentially with hG_{17}, antisera raised against an unrelated peptide and hG_{17}.

Figure 2: illustrates the stomach acid secretion over time or a rat injected sequentially with hG_{17}, hG_{17} premixed with anti-hG_{17}antisera; and hG_{17}.

Figure 3: illustrates the stomach acid output over, time in a rat actively immunized against G_{17} in response to an injection of pentagastrin ("pG") followed by injections of G_{17}.

Figure 4: illustrates the stomach acid output overt time in a rat actively immunized against G_{17} in response to an injection of G_{17} followed by injections of pentagastrin and G_{17}.

Figure 5: illustrates the stomach acid output over time in a control rat in response to the sequential injection of pG, G_{17} and pG.

Figure 6: illustrates stomach acid output over time in a rat actively immunized against G_{17} in response to sequential injections of G_{34}, G_{17} and G_{34}.

Figure 7: illustrates stomach acid output over time in a control rat in response to sequential injections of G_{34}, G_{17} and G_{34}.

Figure 8: depicts the binding capacity in picograms ("pg.") of Antigen per microliter ("μl") of sera of anti-G_{17} antibodies induced by two synthetic peptide antigen epitope polymers of the invention.

Figure 9: illustrates the total quantity of stomach acid secreted by hG_{17}-immune rats and non-immune rats in response to graded doses of hG_{17}. Two non-immune rats and four hG_{17}-immune rats were injected with 0.12 micrograms ("μg") and 2.5 μg doses of hG17. Two hG_{17}-immune rats were injected with doses of 25 μg and 250 μg of hG_{17}.

Figure 10: depicts the stimulation of the growth of human colon cancer cell line, HCT 116, by pentagastrin.

Figure 11: illustrates the effect of anti-G_{17} antibodies on the growth of colon cancer implants in nude mice infused witch G_{17}, as measured by mean tumor volume in cubic milimeters. Group I consisted of rats injected with rat anti-G_{17} antibodies, and Group II consisted of rats injected with normal rat antibodies.

Figure 12: illustrates the effect of anti-G_{17} on the growth of colon cancer implants in nude mice as measured by mean tumor volumes in cubic milimeters. Group 1 was injected with both G_{17} and anti-G_{17} antibodies. Group III was injected only with saline.

Description of the Invention

Since the different forms of gastrin vary in function, it is necessary to selectively neutralize specific forms of gastrin to control specific functions. To regulate gastrin-mediated secretion of stomach acid following meals (the principal source of excess stomach acid relating to ulcers), an immunogen must specifically target G_{17}. In order to selectively neutralize G_{17}, one or more antigenic epitopes on G_{17} that are not found on G_{34} or cholecystokinin, which exhibits carboxy terminal homology with gastrin, must be identified. As discussed above even though G_{17} and the C-terminus of G_{34} are almost identical, the N-terminus of G_{17} is very different from that of G_{34}. This results in antigenic epitopes that are unique to G_{17} and can be separately targeted. We have identified and mapped such a unique epitope on G_{17}. The present invention concerns immunogens comprising this unique epitope. These immunogens result in high levels of anti-G_{17} antibodies that do not crossreact with G_{34}. Thus they block some or all of G_{17} stimulation of gastric acid secretion, while still allowing G_{34} and CCK, which share with G_{17}a common receptor, to carry out their physiologic function.

Our immunoneutralizing approach has several attractive advantages over current treatments for peptic ulcer. One of these advantages is the overcoming of the major problem of patient compliance, since a daily dose of a drug is not required. This invention treats ulcers by preventing the release of excess stomach acid, unlike antacids that neutralize secreted acid. By administering our synthetic peptide as an immunogen, the frequency and quantity of treatment administration is decreased, while at the same time long-lasting control of acid production, long term prevention of recurrence, and reduced side effects and easier patient administration are provided. Unlike conventional anti-ulcer drugs, antibodies generated by the peptide immunogens are very specific to their target. They do not cross the blood-brain barrier, and their use avoids certain complications encountered with drugs, for example, liver toxicities associated with H_2 antagonists.

The immunogens against "little gastrin", or G_{17}, are constructed to induce a selective and specific antibody response to G_{17} in the immunized human or other vertebrate, but not to G_{34} or CCK. This selective immunization to produce G_{17} specific antibodies is crucial to avoid producing antibodies specific for or cross reactive with G_{34}, which might during the treatment of a specific condition induce undesirable side effects by blocking G_{34} physiologic functions. The antibodies resulting from the immunization with such immunogens target the chemical structure of G_{17} which is antigenically and immunogenically unique from the structure of G_{34}.

Peptides comprising the amino acid residues beginning from the amino terminus (amino acid residue number one) of G_{17} and extending up to and including amino acid residue number 12 having the sequence pyro-Glu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr, are used to prepare the immunogens of the invention by coupling them to an immunogenic carrier. The immunogens may contain a part or all of this sequence. The last 5 carboxy-terminal end amino acids of the G_{17} chemical structure (residues 13-17) are not used, because this sequence is a common antigenic sequence between G_{17}, G_{34}, and at least one

other hormone, cholecystokinin (CCK). Fragments of this 12 amino acid sequence of G_{17} may be used.

The most preferred peptide used in the invention is the hexamer pGlu-Gly-Pro-Trp-Leu-Glu-. Other peptides such as pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu, pGlu-Gly-Pro-Trp-Leu-and pGlu-Gly-Pro-Trp are also preferred. One or more other amino acids may also be substituted for those of the natural sequence, so that increased or decreased binding capacity, specificity and/or titer of the antibody response against G_{17} may be induced in the vaccinated host by the immunogen.

In other embodiments of the invention, the use of preformed G_{17} specific polyclonal and/or monoclonal antibodies and their derivatives or fragments produced by immunization, hybridoma, recombinant DNA or other technologies as a method of passive immunization for the control of gastric acid secretion stimulated by G_{17} may be used.

The peptides and immunogens may be produced by any process commonly used in the art including, for example, standard peptide synthesis technologies; methods employing recombinant DNA and associated technologies; antigen mimicking methods including antibody-internal image technology and any other related methodologies that produce a structure that immunologically resembles the antigenic structures of (mimotopes).

The means by which anti-gastrin antibodies prevent acid release has not been thoroughly established. Without being bound by theory, we believe that the acid suppressive effect of our immunogen is due to the binding of antigastrin antibodies to G_{17} in the blood, thereby preventing the binding of gastrin to its physiological receptors on the surfaces of parietal cells.

Thus, gastrin is prevented from signaling parietal cells to secrete acid into the stomach.

In the immunogens of this invention, the epitopic peptides are chemically coupled to "carriers".

Examples of carriers for this purpose include: diphtheria toxoid, tetanus toxoid, keyhole limpet hemocyanin, bovine serum albumin, etc. Fragments of these carriers, including single epitopes, may also be used. Any method of chemically coupling the epitopes to the carriers may be followed. A preferred method utilizes the bifunctional linking agent EMCS described in U.S. Patent 4,302,386; Lee et al., 1981.

The epitopes can be alternatively rendered immunogenic by crosslinking (e.g., polymerizing) a number of epitopes. For this purpose, it may be necessary to extend the molecule bearing the G_{17} epitope by the addition of selected compounds that provide structures through which the crosslinking will occur. These additions must not disrupt the structure of the G_{17} epitope. because the capacity to induce anti-G_{17} antibodies would be lost. For example, to the carboxy terminal end of the exemplified G_{17} epitopic peptide pyro-Glu-Gly-Pro-Trp-Leu-Glu-Glu is added the amino acid sequence Lys-Arg-Pro-Pro-Pro-Pro-Lys, to give pyro-Glu-Gly-Pro-Trp-Leu-Glu-Glu-Lys-Arg-Pro-Pro-Pro-Pro-Lys, which is then polymerixed with glutaraldehyde, which crosslinks the lysine residues, to form the crosslinked immunogen. This crosslinked immunogen should induce specific antibodies against G_{17}.

The immunogens of this invention are therefore useful for more than just the treatment or prevention of peptic ulcers. The immunogens may be used to treat any disease in which the gastrin stimulated secretion of stomach acid or stimulation of the growth of cancer by gastrin (e.g. colorectal and gastric) is a factor.

Administration of these immunogens, compositions containing them, or pharmaceutically acceptable and immunologically effective derivatives thereof, may be via any of the conventionally accepted modes of administration of agents which exhibit immunogenicity.

The compositions in which the immunogens are administered may be in a variety of forms. These include, for example, solid, semi-solid and liquid dosage forms, such as powders, liquid solutions or suspensions, suppositories, and injectable and infusible solutions. The preferred form depends on the intended mode of administration and therapeutic applications. The compositions also will preferably include conventional pharmaceutically acceptable carriers and may include other medicinal agents, carriers, adjuvants, excipients, etc., e.g. human serum albumin or plasma preparations. Preferably, the compositions of the invention are in the form of a unit dose. The amount of active compound administered as an immunization or as a medicament at one time, or over a period of time, will depend on the subject being treated, the manner and form of administration, and the judgment of the treating physician. However, an effective dose may be in the range of from about 1 μg to about 10 mg of the immunogen of this invention, preferably about 100 μg to about 2 mg; it being recognized that lower and higher doses may also be useful.

This invention provides a novel immunological approach to the treatment of tumors whose growth is dependent upon or stimulated by one or more of the forms of gastrin. According to the invention, antibodies are induced in the patient by passive or active immunization with immunogens that target G_{17}. Such antibodies will bind to and neutralize G_{17}, thereby preventing this hormone from acting upon the tumor. The antibody-mediated deprivation of such hormonal activity thus constitutes a means of controlling gastrin dependent tumors.

Similarly, this invention additionally provides a means of treatment for the effects of tumors that produce G_{17}, thereby preventing pathological consequences resulting from abnormally elevated levels of hormone.

This invention also provides a method for selectively reversing the antibody-mediated immunity induced by the anti-G_{17} immunogens of the invention. Soluble monovalent, monodeterminant epitopic peptides are injected into the patient to bind to and neutralize the selected anti-hormone antibodies. The antibodies will then be incapable of binding additional quantities of the G_{17}, and will no longer affect the hormone's biological activity.

This example demonstrates a means of preparing immunogens to induce anti-G_{17} antibody responses.

EXAMPLE 1

Peptides for the induction of specific immune responses to G_{17} were prepared by standard solid state synthesis methods. Each peptide was characterized as to amino acid content and purity.

Peptides with the following amino acid sequences (including the sequence of the C-terminal spacer) were synthesized:

Peptide 1- Human G_{17}(1-6) ("hG_{17}(6)"): pGlu-Gly-Pro-Trp-Leu-Glu-Arg-Pro-Pro-Pro-Pro-Cys

Peptide 2- Human G_{17}(1-5) ("hG_{17}(5)"): pGlu-Gly-Pro-Trp-Leu-Arg-Pro-Pro-Pro-Pro-Cys

Peptide 3- Human G_{17}(1-4) ("hG_{17}(4)"): pGlu-Gly-Pro-Trp-Arg-Pro-Pro-Pro-Pro-Cys

Peptide 4- Rat G_{17}(1-6) Leu 5 ("rG_{17}(6)Leu 5"): pGlu-Arg-Pro-Pro-Leu-Glu-Arg-Pro-Pro-Pro-Pro-Cys

Each of these peptides was conjugated to amino groups present on a carrier such as diphtheria toxoid ("DT") via the terminal peptide cysteine residue, utilizing hetero-bifunctional linking agents containing a succinimidyl ester at one end and maleimide at the other end of the linking agent.

To accomplish the linkage between any of Peptides 1-4 above and the carrier, the dry peptide was dissolved in 0.1M Sodium Phosphate Buffer, pH 8.0, with a thirty molar excess of dithiothreitol ("DTT"). The solution was stirred under a water-saturated nitrogen gas atmosphere for four hours. The peptide containing reduced cysteine was separated from the other components by chromatography over a G10 "Sephadex" column equilibrated with 0.2M Acetic acid. The peptide was lyophilized and stored under vacuum until used. The carrier was activated by treatment with the hetero-bifunctional linking agent e.g. Epsilon-maleimidocaproic acid N-hydroxysuccinimide ester, ("EMCS"), in proportions sufficient to achieve activation of approximately 25 free amino groups per 10^5 molecular weight of carrier. In the specific instance of diphtheria toxoid, this amounted to the addition of 6.18 mg of EMCS (purity 75%) to each 20 mg of diphtheria toxoid.

Activation of diphtheria toxoid was accomplished by dissolving each 20 mg aliquot of diphtheria toxoid in 1 ml of 0.2 M Sodium Phosphate Buffer, pH 6.45. Aliquots of 6.18 mg EMCS were dissolved into 0.2 ml of Dimethyl Formamide ("DMF"). Under darkened conditions, the EMCS was added dropwise in 50 microliter ("μl") amounts to the DT with stirring. After 2 hours of incubation in darkness, the mixture was chromatographed on a G50 "Sephadex" column equilibrated with 0.1 M Sodium Citrate buffer, pH 6.0, containing 0.1 mM EDTA.

Fractions containing the EMCS-activated diphtheria toxoid were concentrated over a PM 10 ultrafiltration membrane under conditions of darkness. The protein content of the concentrate was determined by either the Lowry or Bradford methods. The EMCS content of the carrier was determined by incubation of the activated carrier with cysteine-HCl followed by reaction with 10 mM of Elman's Reagent, 5,5'-dithio-bis(2-nitrobenzoic acid), 10mM. The optical density difference between a blank tube containing cysteine-HCl and the sample tube containing cysteine-HCl and carrier was translated into EMCS group content by using the molar extinction coefficient of 13.6 x 10^3 for 5-thio-2-nitrobenzoic acid at 412 nm.

The reduced cysteine content (-SH) of the peptide was also determined utilizing Elman's Reagent. Approximately 1 mg of peptide was dissolved in 1 ml of nitrogen gas-saturated water and a 0.1 ml aliquot of this solution was reacted with Elman's Reagent. Utilizing the molar extinction coefficient of 5-thio-2-nitrobenzoic acid (13.6 x 10^3), the free cysteine -SH was calculated. An amount of peptide containing sufficient free -SH to react with each of the 25 EMCS activated amino groups on the carrier was dissolved in 0.1M Sodium Citrate Buffer, pH 6.0, containing 0.1 mM EDTA, and added dropwise to the EMCS-activated carrier under darkened conditions. After all the peptide solution had been added to the carrier, the mixture was incubated overnight in the dark under a water-saturated nitrogen gas atmosphere.

The conjugate of the peptide linked to the carriers via EMCS was separated from other components of the mixture by chromatography over a G50 "Sephadex" column equilibrated with 0.2 M Ammonium Bicarbonate. The conjugate eluted in the column void volume was lyophilized and stored desiccated at -20 °C until used.

The conjugate may be characterized as to peptide content by a number of methods known to those skilled in the art including weight gain, amino acid analysis, etc. Conjugates of Peptides 1-4 and diphtheria toxoid produced by these methods were determined to have 20-25 moles of peptide per 10^5 MW of carrier and all were considered suitable as immunogens for immunization of test animals.

EXAMPLE 2

As examples of the utilization of peptides containing sequences of human gastrin as immunogens to induce immune responses against hG_{17} or hG_{34}, we have immunized rats with the conjugate immunogens constructed from Peptides 1-4 of Example 1 and diphtheria toxoid ("DT") (referred to as Immunogens 1-4, respectively).

Six different groups of 15 Sprague-Dawley female rats (200 g. body weight) were each immunized with one of the immunogens constructed from Peptides 1-6. Each animal was injected subcutaneously with 0.25 ml of immunogen consisting of 0.1 mg of conjugate dissolved in 0.125 ml of 0.1 M Sodium Phosphate Buffered Saline, pH 7.3, emulsified with an equal volume of Squalene-"Arlacel" (4:1 ratio volume/volume) vehicle containing 0.05 mg of Nor MDP as adjuvant.

Two additional groups of 15 rats were immunized with a peptide-DT conjugate in which the peptide had no sequence homology with gastrins so as to act as a negative immunization control.

Each rat was given an injection of immunogen at 0, 3, and 6 weeks. Blood was collected from each rat at 3, 6, and 8 weeks of the experiment. Serum was collected from each blood sample and stored at -20°C until utilized in assays to determine the presence of anti-gastrin antibodies.

Two types of assays were used to detect antigastrin antibodies. A solid-phase enzyme linked immunosorbent assay (ELISA) and a liquid phase radioimmunoassay (RIA) were employed.

ELISA was used to screen for reaction or cross reaction of antisera raised against Peptides 1-4 with Peptides 1-4 or with hG_{17} or hG_{34}, or hCCK. The RIA was used to quantitate the antibody levels in the antiserum of each immunized animal that was reacted with hG_{17} or hG_{34} by determining the antigen binding capacity (ABC), expressed as pg hormone bound per ("µl") of antiserum (pg/µl).

The ELISA was conducted by coating polystyrene 96 well plates (Immulon II) with 1 µg/ml of Peptides 1-4, hG_{17} or hG34, Serial dilutions of test antisera of 1 x 10^{-1} to 1 x 10^{-8} were incubated with each test peptide for 30 minutes at room temperature. In some instances antisera raised against a particular peptide of the Peptides 1-4 were preincubated with large excesses of the other peptides of the Peptide 1-4 group or with hG_{17} or hG_{34} in an attempt to inhibit binding of the antiserum to its particular peptide and also to demonstrate the occurrence of antibodies in the antisera that were specific for the sequence (spacer) of each peptide that was common to all of Peptides 1-4 (i.e. Arg-Pro-Pro-Pro-Pro-Cys). After washing each well thoroughly to remove unbound antibody, each well was treated with biotinylated anti-rat immunoglobulin reagent for 30 minutes at room temperature. After another wash sequence to remove unbound anti-rat reagent, avidin-alkaline phosphatase conjugate was added and the mixture was incubated for an additional 30 minutes. The mixture was washed thoroughly to remove unbound avidin-alkaline phosphatase reagent, and the chromogenic substrate PNPP was added for a 10 minute period. The absorbance of each well was read at 490 nm after the 10 minute incubation.

The standard RIA procedure was followed. In the RIA, 0.1, 1.0 or 10.0 µl aliquots of antiserum were incubated with approximately 200 pg of ^{125}I labeled hG_{17} or 400 pg of labeled hG34. The antisera were incubated with label for 2 hours, followed by a precipitation of hormone-antibody complexes with 25% polyethylene glycol. Antigen binding capacities for each antiserum where then determined from the amount of radioactive hormone precipitated. To demonstrate the specificity of the reaction of the ^{125}I labeled hormone with the antisera, aliquots of the antisera were preincubated in some tests with excess amounts of the hormone that were not labeled with ^{125}I to inhibit binding of the antisera to the labeled hormone.

The specificities of the antibody responses induced by Immunogens 1-4 as measured by ELISA are depicted in Table 1. Immunogen 1, containing the peptide sequence of hG_{17} (1-6), induced antibodies that reacted strongly with hG_{17} and hG_{17} (1-6) peptide, but not with hG_{34}.

Immunogens 2 and 3 induced antibody responses specific for hG_{17} that were much weaker than those induced by Immunogen 1 (Table 1). Inhibition experiments, demonstrated that the weak reactivities of anti-Imunogen 2 and 3 antibodies for Peptides 1-4 are specific for the common spacer sequence of Peptides 1-4.

Immunogen 4, containing the rat G_{17} sequence, induced antibodies that weakly reacted with Peptides 1-6, but not hG_{17} or hG_{34} (Table 1). Inhibition experiments demonstrated that these antibodies were directed against the spacer sequence common to Peptide 1-4.

Table 1

Antisera to:	Reaction in ELISA with: hG$_{17}$	Peptide 1 hG$_{17}$(1-6)	Peptide 2 hG$_{17}$(1-5)	Peptide 3 hG$_{17}$(1-4)	Peptide 4 rG$_{17}$(1-6)	hG$_{34}$
Immunogen 1 hG$_{17}$(1-6)-DT	++	+++	+	+ -	+ -	0
Immunogen 2 hG$_{17}$(1-5)-DT	+	+ -	+	+ -	+ -	0
Immunogen 3 hG$_{17}$(1-4)-DT	+ -	+ -	+ -	+	+ -	0
Immunogen 4 rG$_{17}$(1-6)-DT	0	+ -	+ -	+ -	+	0

+++ to +: Strongly reactive   + -: Weekly reactive   0: No reaction

All antisera were also tested against hCCk; none of the antisera bound to hCCK.

Table 2 demonstrates the RIA-measured antigen binding capacities ("ABC") versus hG$_{17}$ or hG$_{34}$ of antisera raised against Immunogens 1-4 after three immunizations of rats with 0.1 mg of conjugate.

Table 2

Rats Immunized With:	Mean RIA ABC (pg/μl)	
	hG_{17}	hG_{34}
Immunogen 1 hG_{17}(1-6)-DT	19.29	0.00
Immunogen 2 hG_{17}(1-5)-DT	7.59	0.00
Immunogen 3 hG_{17}(1-4)-DT	2.15	0.00
Immunogen 4 rG_{17}(1-6)-DT	0.00	0.00

The liquid phase RIA demonstrated that Immunogens 1-3 containing the hG_{17} peptide sequence induced antibodies that reacted only with hG_{17}.

The ELISA and RIA assays thus demonstrate the specificity of the responses to hG_{17} or hG_{34} that are induced by Immunogens 1-4.

EXAMPLE 3

This example demonstrates the ability of antisera raised against Peptide 1 (hG_{17} (1-6)) to neutralize the in vivo acid-stimulating activity of hG_{17}. In this demonstration an amount of hG_{17} is mixed with an excess amount of anti-Peptide 1 antiserum sufficient to bind to all the hG_{17} prior to injection of the complex into a normal (non-immunized) rat.

In control experiments, the amount of hG_{17} sufficient to stimulate an increase of acid secretion of at least 100% above non-stimulated acid secretion in normal rats was determined to be 0.4 μg of hG_{17} hormone per kg body weight.

Antisera from the rats immunized with Immunogen 1 were pooled and standard amounts of antisera were incubated with 200 pg ^{125}I labeled hG_{17} after incubation with increasing amounts of cold hG17 as inhibitor. Based on this inhibition study, 1 ml of antiserum was capable of binding 1000X the 0.4 μg/kg dose of hG_{17} to be administered to rats. As a safety factor, the 0.4 μg/kg (approximately 120 ng) of hormone was mixed with 2.5 ml of anti-hG_{17} specific antiserum raised against Immunogen 1.

Rats to be injected with hG_{17} complexed with anti-hG17 antibodies were surgically prepared for collection of stomach secretions by the perfused rat stomach procedure.

Under general anesthesia and following tracheostomy, the rat was cannulated via the esophagus and duodenum to allow continuous perfusion of the stomach with 0.9% saline. The stomach perfusate was collected as 5 minute interval samples and was titrated for acid content by neutralization with base (sodium hydroxide). Incremental and total acid input during the duration of the experiment and after each treatment was determined.

Each control or experimental test rat was first injected with 0.4 μg/kg hG_{17} to determine the rats total acid secretory response to this treatment. The first treatment was followed one hour later in test rats with an injection of 0.4 μg/kg of hG_{17} that had been premixed for one hour with 2.5 ml of anti-hG_{17} specific antiserum. Control rats received an injection of hG_{17} mixed with 2.5 ml of antiserum raised against an unrelated peptide. After one hour, a second injection of free hG_{17} was administered to the test and control rats; and stomach perfusate was collected for an additional hour. The total acid outputs induced by the second and third injections of hG_{17} were expressed as a percentage of the total acid output induced by the first injection of hG_{17}.

In five rats tested by this experimental procedure there was an 81%-100% (mean = 94%) reduction in the acid secreted by the perfused rat stomach in response to the hormone premixed with anti-hG_{17} specific antibody (second injection) or to the third injection consisting of free hG_{17} alone. Control rats experienced little or no reduction in acid secretion stimulated by the second and third injections of hormone. Figure 1 and Figure 2 illustrate the responses of a control rat (Figure 1) and experimental rat (Figure 2) to these treatments.

EXAMPLE 4

A major application of this invention is the active immunization of humans to induce specific immunity against G_{17} for ulcer therapy and prevention. In this example, it is demonstrated that active immunization with an anti-G_{17} immunogen induces antibodies that dramatically suppress G_{17}-mediated release of stomach acid.

To actively immunize rats against G_{17}, we follow the methods used to obtain antisera in the passive immunization tests as described in Example 3. An immunogen consisting of the hG_{17} (1-6) peptide covalently coupled to diphtheria toxoid (DT) is prepared as described in Example 1. This immunogen is suspended in Phosphate Buffered Saline at a concentration of 4.0 mg/ml. The antigen is emulsified in squalene:"Arlacel" (4:1) vehicle, at a final ratio of 1:1 (antigen vehicle). Nor-MDP is included in the mixture to give a final concentration of nor-MDP of 200 µg/ml. The final concentration of the DT-hG_{17}(1-6) in the formulation is 2.0 mg/ml. Experimental rats are injected with 0.25 ml of this preparation intraperitoneally. Each injection thus delivers approximately 500 µg of immunogen plus 50 µg of nor-MDP. A second injection is similarly administered 21 days later.

Blood samples for antibody analysis are obtained by tail vein bleeding before the first injection and 14 days after each injection. Sera are prepared by allowing the blood to clot for 30 minutes at room temperature followed by centrifugation at 400 x g to remove the clots. The sera are stored frozen until used.

To determine the antibody responses of the immunized rats, a RIA is employed as described in Example 2. The results of this test show that the immunization procedure induces high titers of antibody against G_{17}. These responses are specific for G_{17}; no reactivity is detected with G_{34}, with pentagastrin (the biologically active, carboxy terminal fragment of G_{17}, G_{34}, and CCK), or with CCK. The antibodies are thus directed against the unique epitope on G_{17} that is selectively targeted by the immunogen. These results are similar to those of Example 2.

The use of the immunogens described herein for the active immunogen is not limited to the adjuvant, vehicle, injection schedule, etc., described above. Any means of safely inducing immunity against G_{17} using the immunogens described can be applied. This includes using alternative dosages, routes, vehicles, adjuvants, exipients, slow-release compounds, etc.

We test for the neutralization of G_{17}'s biological activity in the immunized animals using the perfused rat stomach method, as described in Example 3, with the important difference that we do not inject antisera into the rats (passive immunization) because the actively immunized rats are making their own antibodies against G_{17}. The dosages of compounds administered in these tests, with delivery times of 5 minutes per total dose, are: G_{17} = 0.4 µg/kg, G_{34} = 0.8 µg/kg, and pentagastrin = 2.0 µg/kg. Stomach contents sampling times are 5 minutes per sample. The stomach acid outputs are calculated as the percent of maximal acid output

$$= (100)\ \frac{An - Ab}{Amax - Ab},$$

where An = the acid produced over each 5 minute sampling interval (as determined by titration with NaOH); Amax = the maximal 5 minute release of stomach acid upon stimulation, usually (but not necessarily) by pentagastrin; and Ab = the baseline level of acid present at the time of a given stimulation (with G_{17}, pentagastrin, or G_{34}).

The effects of active immunization against G_{17} upon the G_{17} and pentagastrin ("pG")-induced acid secretion are shown in Figures 3 and 4. The ordinate represents the percent of acid output compared to the maximal acid output induced by pentagastrin. These experiments differed, by design, in the order of G_{17} and pentagastrin challenge. In both cases, it is clear that in the G_{17} immunized rats the production of stomach acid in response to G_{17} (Figure 3, Peaks 2 and 3; Figure 4, Peaks 1 and 3) is substantially reduced in comparison with acid secretion induced by pentagastrin (Figure 3, Peak 1; Figure 4, Peak 2). The mean reduction in the total G_{17}-mediated acid secretion in our G_{17} immune rats is 85% (compared to pentagastrin).

We verified that the acid reductions were a direct consequence of immunization against G_{17} by conducting challenges with G_{17} or pentagastrin in control rats. The control animals were immunized in an identical manner as the G_{17}-immune rats, except that the controls received antigen consisting of DT conjugated to an unrelated peptide (i.e., non-crossreactive with gastrin). RIAs and ELISAs, run on sera from these animals, demonstrated that they produced high antibody titers against both DT and the unrelated peptide, but none against G_{17}, pentagastrin, G_{34} or CCK. When tested for acid secretion, the control rats responded equally well to challenges with both G_{17} and pentagastrin. The results of such a test are shown in Figure 5. This rules out the remote possibility that the neutralization of G17 in the G_{17}-immune rats was caused by non-specific factors (e.g., adjuvant effects, cross-reactive anti-DT antibodies, etc.).

A technical challenge presented by the perfused rat stomach assay was the selection of the appropriate acid stimulatory compound for use as a positive control. The exquisite specificity for G_{17}'s unique epitope

that is characteristic of antibodies induced by our immunogen enabled us to use the ideal control compound: pentagastrin. Pentagastrin comprises the receptor binding/stimulatory sequence of G_{17} and also of both G_{34} and CCK, and it is not bound by antibodies induced by our immunogen. The responses to pentagastrin demonstrated that our immunized animals' acid response mechanism to G_{17} stimulation were functional. In addition, the pentagastrin responses established the level of acid secretion to be expected from G_{17} stimulation. The dosages of G_{17} and pentagastrin, which we determined experimentally, were selected to induce approximately equal acid secretory responses in control rats (see Figure 5). Thus, we were able to accurately quantitate reductions in acid secretion resulting from the neutralization of G_{17}.

For completeness, we have also challenged with G_{34}. We designed our immunogen to specifically neutralize G_{17}-mediated acid secretion (particularly following food intake) and to have no effect upon acid output induced by G_{34} (which provides for basal stomach activity). Since the antisera from the G_{17} immune rats do not react with G_{34}, we expected to see no effect upon G_{34}'s ability to stimulate acid secretion. Indeed, as shown in Figure 6, the immunized rats secreted normal quantities of acid in response to G_{34} stimulation (Peaks 1 and 3). As expected, the injection of G_{17} failed to induce acid secretion in these animals (Figure 6, Peak 2). Both G_{17} and G_{34} induce strong acid secretory responses in control rats (immunized against an irrelevant peptide), as can be seen in Figure 7. Clearly, the anti-G_{17} antibodies induced by our immunogen have no effect upon the functions of other molecules to which the antibodies do not bind. The hG_{17}(1-6)-based immunogen described herein induces antibodies that are specific for G_{17} and neutralize G_{17}'s acid-releasing activity. Such an immunogen should thus protect against and cure peptic ulcers.

EXAMPLE 5

This example demonstrated that a polymerised peptide immunogen can be constructed and used to reduce anti-G_{17} antibody responses. Synthetic peptides have been produced that contain the unique epitope on G_{17} and in addition carry reactive groups that can be selectively bound to crosslinking agents. These peptides serve as monomers in the construction of a polymer immunogen. By including two or more reactive groups in each peptide it is possible to construct multi-peptide aggregates, or polymers, by reaction of the groups with a cross-linking agent. Such polymers are then used as immunogens to induce antibodies against the G_{17} epitope expressed by the peptide. These antibodies bind to G_{17} in vivo and neutralize G_{17}, thus mediating an anti-ulcer effect. These polymerized peptides have an advantage in that they can be used as immunogens by themselves without a coupling to an immunogenic protein carrier.

The following peptide designated as Peptide 5 was constructed:

```
 1   2   3   4   5   6   7   8   9   10  11  12  13  14
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Lys-Arg-Pro-Pro-Pro-Pro-Lys
```

The G_{17} epitope is contained in amino acids 1-7 of the peptide. Other epitopes, e.g. G_{34} epitopes, can also be used to construct other polymer immunogens according to the invention. Amino acids 8 and 14, which are both Lys, contain amino groups as side groups. These amino groups act as functional groups which are reacted with the functional groups on the crosslinking agent to form the crosslinked peptide polymer. Other amino acids containing side functional groups could be substituted for Lys, depending on the reactivity of the functional group with the group on the crosslinking agent to be used. The location of the functional amino acids can be varied in the peptide as long as they are not positioned within the epitope region. Additional reactive amino acids could also be added to increase crosslinking. These additional amino acids could be reactive with the same or alternative crosslinking agents. It follows that more than one type of crosslinking agent can be used.

Amino acids 9-13 comprise a "spacer region" between the reactive amino acids 8 and 14. The composition, number of amino acids and length of the spacer can be varied. If desirable, helper T-cell epitopes can also be included in the peptide.

Peptide 5 was synthesized and purified by standard solid phase peptide synthesis and purification methodologies. Any other method of peptide production well known to those skilled in the art including recombinant DNA technology can also be used to produce the peptides of the invention.

5.0 mg of the peptide was dissolved in 1.0 ml phosphate buffer (0.1M; pH=6.8). To this was added glutaraldehyde (Grade 1, Sigma Chemical Co.) in a 2:1 molar ratio of glutaraldehyde to peptide. The glutaraldehyde was added dropwise with stirring, at room temperature.

The reaction was allowed to proceed overnight, at room temperature, with stirring. 50.0 mg. of sodium borohydride were then added slowly to the reaction mixture, and the mixture was stirred at room temperature for an additional hour. The mixture was transferred to dialysis tubing, 1,000 molecular weight cutoff (#132636, Spectrum Medical Industries, Inc.), and exhaustively dialyzed against saline. The peptide-polymer was stored frozen at -20 °C.

The polymer was analyzed by SDS-PAGE using a 15% polyacrylamide gel. The electrophoresis demonstrated that the polymerized peptide contained polymers of various sizes comprising multiples of the peptide. The average polymer contained 6 peptides; however, the size of the polymers ranged up to 12 peptides per molecule.

A second polymer was made using the identical procedures, except that a 20:1 molar ratio of glutaraldehyde to peptide was used. The SDS-PAGE analysis of the second polymer gave similar results with respect to the size range as compared to the 2:1 polymer.

Each polymer preparation, 2:1 and 20:1, was used to immunize two separate groups off five mice per group. Prior to injecting the mice with polymer, blood samples were taken from each mouse. The preparation was suspended in Freund's Complete Adjuvant ("FCA") H37Ra (DIFCO Labs) in a 1:1 (vol:vol) ratio of polymer:FCA. The mice were each injected intraperitoneally with 100 μg polymer in 0.2 ml of the mixture. After 21 days each mouse was given a second injection off the same polymer with which it had been injected previously. In the second injection, the antigen was administered intraperitoneally in saline, at 100 μg per mouse. Each mouse was bled 14 days after the second injection and the sera were isolated. The mouse sera were assayed for anti-G_{17} antibodies by radioimmunoassay (RIA). 1.0 μl of sera was added to 300 μl of buffer (1% BSA in phosphate buffered saline with 0.005M EDTA, pH = 7.2). To each of these samples was added 100 μl or 3000 CPM of ^{125}I-labeled G_{17} (NEN, Specific activity = 12 μCi/ug). The samples were incubated 1.0 hour at room temperature. We next added 100 μl of Calf Serum (Hyclone Labs), immediately followed by 500 μl of 25% polyethylene glycol-8000 (Sigma). The samples were mixed and then centrifuged for 30 minutes at 500 x g at room temperature. The supernatant was discarded, and the pellet suspended in 250 μl of saline at 90 °C. The suspension was transferred to 3.0 ml of Scintiverse II [Fisher Scientific] in mini-vials for liquid scintillation counting. The samples were counted in a Beckman Liquid Scintillation counter (#LS 5000 LE) for ^{125}I. The binding capacities off the antisera were calculated from the resulting ^{125}I counts per sample and are depicted in Figure 8.

Both of the polymers induced anti-G_{17} antibody responses. Polymer #1, the 2:1 ratio polymer (Figure 8), induced a very strong response off 56 pg of antigen bound per μl off sera. Polymer #2, the 20:1 ratio polymer (Figure 8), induced a response that was 10-fold lower. The response induced by polymer #1 is equivalent to that induced by three injections of the hG_{17}(1-6)-DT immunogen of Example 2 in rats.

Thus, polymerized synthetic peptides can be used to induce potent anti-G_{17} antibody responses.

EXAMPLE 6

The following example demonstrates that anti-G_{17} antibodies neturalize the tumor-stimulatory activity of G_{17} in vitro.

HCT-116 cells (a human colon cancer cell line) were cultured in McCoy's 5a medium (McCoy et. al., Proc. Soc. Exper. Biol. Med. 100:115-118) supplemented with epidermal growth factor (10 ng/ml), insulin (20 μg/ml), transferrin (4 μg/ul), sodium selenite (10^{-8}M), hydrocortisone (2 μg/ml), and triiodothyronine ($4X10^{-10}$M). Subcultures were made once weekly for four weeks by treating cultures with 0.5% Trypsin + 0.2% EDTA in Hanks Balanced Salts Solution to remove adherent cells, followed by inoculation of T-75 tissue culture flasks with approximately $1x10^6$ cells. Cultures were maintained under standard conditions (37 °C, 100% humidity, 5% CO_2).

Prior to testing, the HCT-116 cells were synchronized to late G_0 phase with thymidine, as follows: the HCT-116 cells were seeded into 24 well culture plates at approximately $1X10^4$ cells per well and incubated overnight in 1 ml supplemented McCoy's medium 5a. The medium was then removed and replaced with fresh supplemented medium. Thymidine was added to 0.8 mM final concentration to each well and the cultures were incubated for 24 hours. At the end of the synchronization period, the medium containing thymidine was replaced with test media as described below.

To demonstrate that HCT-116 cells proliferate under the influence of gastrin, the synchronized HCT-116 cells were grown in supplemented McCoy's 5a medium, in the presence or absence of 10 μM pentagastrin (the hormonally active segment of gastrin). To assess cell proliferation, total cell counts were performed after selected incubation times. As shown in Figure 10, HCT-116 cells proliferated more rapidly in the presence of pentagastrin than in the absence of the hormone. This difference was already evident after three days of culture, and was maximal by day five. The increase in the number off cells of pentagastrin-

treated culture at the end of eight days was three times greater than that off the non-pentagastrin-treated cultures.

To demonstrate that anti-G_{17} antibodies neutralize the proliferative activity of G_{17}, the effect of anti-G_{17} antisera upon G_{17}-induced increases in the rate of [^{3}H]-thymidine uptake by HCT-116 cells was studied.

Synchronized HCT-116 were cultured in 24 well plates of 10^4 cells/well in supplemented McCoy's 5a medium without FBS. The total culture volume was 1 ml per well. Four wells were cultured for each test condition. Cells were grown in presence of G_{17} at two concentrations, 5 μM and 50 μM. At each G_{17} concentration, rat anti-human G_{17} antisera (antigen binding capacity determined by RIA= 30 pg/μl or normal rat sera were added at a final dilution of 1:25.

After 8 days of culture, 0.4 μCi of [^{3}H] thymidine (specific activity = 2 Ci/mMole) were added to each test well. Following 16 hours' incubation, the replicates were processed with a Multiple Automated Cell Harvester (Mini-Mash II, Whittaker Bioproducts) and the [^{3}H]-thymidine incorporation was determined by scintillation counting.

As shown in Table 3, the addition of anti-G_{17} antiserum caused a substantial reduction in [^{3}H]-thymidine incorporation, relative to uptake by cells cultured in the presence of normal rat serum. At 5 μM G_{17}, counts incorporated were reduced by 59%; at 50 μM G_{17}, label uptake was reduced by 34%. As [^{3}H]-thymidine incorporation directly reflects cell proliferation, this test shows that anti-G_{17} antiserum inhibits the proliferation activity of human G_{17} on colon cancer cells.

Table 3

Quantity of Human G_{17} in Culture	Mean CPM [^{3}H] Thymidine	Incorporated(+/-SE)
	Normal Rat Serum	Anti-hG_{17} Serum
50uM	2130 ± 141	1404 ± 310
5uM	1621 ± 131	664 ± 206

EXAMPLE 7

The following experiment was performed to demonstrate that the growth of established HCT-116 tumors is retarded when nude mice bearing the tumors are treated with anti-gastrin immunoglobin.

Anti-human G_{17} serum was obtained from rats immunized against hG_{17} ((1-6)-DT (i.e., Immunogen 1). Normal serum was obtained from nonimmuized rats. Immunoglobulin fractions of the anti-G_{17} serum and the normal rat serum were prepared by affinity chromatography, using Protein A Sepharose. Both immunoglobulin preparations were adjusted to a final protein concentration off 1 mg/ml in PBS. Measured by RIA, the G_{17}-direct antigen binding capacity (ABC) of the anti-human G_{17} immunoglobulin preparation was 30 pg/μl. The normal rat immunoglobulin preparation had no anti-human G_{17} activity.

Thirty nude mice were each implanted subcutaneously (dorsally near the left shoulder) by trocar needle (14 gauge) with a single 2 mm cube of HCT-116 tumor tissue. The tumors were allowed to grow for one week prior to random assignment of individual mice to one of three treatment groups, ten mice per group, on day 0 of the Test.

Group I was treated with anti-human G_{17} immunoglobulin function. Each mouse in Group I received intraperitoneal injections of 0.5 mg. of immunoglobulin beginning on day 0 and repeated on days 4, 8, and 14 of the test. The Mean human G_{17} ABC of the mouse sera on day 16 was 8.3 ± 1.3 pg/μl.

Group II was treated with normal rat immuglobulin fraction. Each mouse received intraperitonael injections of 0.5 mg of immunoglobulin beginning on day 0 and repeated on days 4, 9, and 14, of the test. On day 16, the human gastrum G_{17} ABC's of those sera was O pg/ul.

Group III received no immunoglobulin injection but was injected instead with saline on days 0, 4, 9, and 14 of the test. The human gastrin G_{17} ABC's of those sera was O pg./ul. one day 16.

On day 1, mice of Groups I and II were implanted subcutaneously with osmotic pumps (Alzet 2002) that were charged with human G_{17} that delivered 10 μg/day human G_{17} continuously for 14 days. On day 1, mice of Group III were implanted subcutaneously with osmotic pumps charged with saline which delivered a dose of saline instead of hormone continuously for 14 days.

Each mouse was observed daily for changes in the size of their individual tumors. Measurements of the tumors were made by vernier caliper approximately every other day. Volumes of tumors were estimated by the calculation: volume = (length X width^2) ÷ 2 (Euhus et. al., 1986, J. Surg. Oncol. 31:229-234).

Observations on Group II mice were made through day 17 of the test, on which day the Group II mice were euthanized. Observations on Groups I and III were continued until day 32 of the test.

As shown in Figure 11, tumors in Group II mice (administered human G_{17} and treated with normal rat immunoglobulin) grew very rapidly in response to the added gastrin, increasing more than 120 fold in volume in 16 days.

Tumors of Group I mice (administrated human G_{17} and treated with anti-human G_{17} immunoglobulin) grew at a significantly slower rate than the tumors of Group II. By day 16, the volume of the tumors of Group I mice were, on average, approximately 11 times smaller than those of Group II mice (Table 4). The results of this test demonstrate that HCT-116 tumors are stimulated to grow by human gastrin G_{17} and that treatment with anti-human G_{17} specific immunoglobulin neutralizes this growth-promoting effect and significantly slows the growth off HCT-116 tumors.

Tumors of Group III mice (not administered hG_{17} and no immunoglobulin treatment) grew at a faster rate than Group I (Figure 12), suggesting an autocrine production of human G_{17} (by the HCT-116 tumor cells) that stimulated the tumors to grow. Tumor-produced G_{17} would be neutralized in the Group I animals, due to the injected anti-G_{17} immunoglobulins. On day 32 of the test, the Group III tumors had attained approximately the same volume attained by Group II tumors on day 16. However, on day 32 tumors of Group I were significantly smaller than tumors of Group III (i.e., 3.3 times smaller volume, Table 4), indicating an inhibitory effect of the anti-G_{17} immunoglobin on tumor growth.

Table 4

Group	Treatment	Mean Tumor Volume day 16	(mm^3 ± SE) day 32
I	Anti-G_{17} Antibody plus G_{17}	142 ± 38	545 ± 137
II	Normal Rat Serum plus G_{17}	1512 ± 348	-
III	Saline and no G_{17}	391 ± 63	1825 ± 313

EXAMPLE 8

The following test demonstrates that antibodies against human G_{17} inhibit tumor development and growth in nude mice that have been injected with suspended cells of the human colon cancer line, HCT-116.

Anti-human G_{17} serum was obtained from rats immunized against hG_{17}(1-6)-DT. Normal rat serum was obtained from nonimmunized rats. Immunoglobulin fractions of the anti-G_{17} serum and the normal rat serum were prepared by affinity chromatography, using Protein A-"Sepharose". Both immunoglobulin preparations were adjusted to a final protein concentration of 1 mg/ml in PBS. Measured by RIA, the G_{17}-direct antigen binding capacity (ABC) of the anti-human G_{17} immunoglobulin preparation was 30 pg/µl. The normal rat immunglobulin preparation had no anti-human G_{17} activity.

HCT-116 cells were grown in vitro in the presence of pentagastrin as described in Example 6. Cells in the log phase of growth were collected from in vitro culture, washed by centrifugation in PBS, and resuspended to 10 cells/ml. Viability was assessed by trypan blue exclusion.

Each of 20 nude mice (NIH strain) were injected subcutaneously on their dorsal side near the right shoulder with a single bolus of $5x10^6$ cells.

Two days after the injection of HCT-116 cells, the mice were randomly assigned to two groups of ten mice each. One group (Treated Group) was injected intraperitoneally with 0.5 mg per mouse of the anti-human G_{17} immunoglobulin preparation. The other group (Control Group) was treated with 0.5 mg per mouse of the normal immunoglobulin fraction Seven days later these treatments were repeated. These treatments resulted in sera G_{17} binding capacities of 11.1 pg/µl in the anti-G_{17} immunoglobulin treated mice and 0 in the normal immunoglobulin-treated mice (See Table 5).

Two days after injections off the HCT-116 cells, all of the mice were started on daily injections of human G_{17} for 16 consecutive days. A total daily dose of 51 µg of hormone per mouse was administered in 3 separate injections of 17 µg each, given at 4 hour intervals. The mice were bled on day 16 of the test to determine binding capacities by RIA.

Daily observations on the occurrence and growth of tumors were made visually and by palpation. On day 18 of the test, the tumors were measured by vernier caliper and the volume of each tumor estimated by the following formula: volume = (length X $width^2$) ÷ 2 (Euhus et. al., 1986, J. Surg. Oncol. 31:229-234).

On Day 18, the mice were euthanized and those mice without visually detectable tumors were dissected and further examined for tumors under a stereo microscope at 10X magnification.

As shown in Table 5, the anti-human G_{17} immunoglobulin prevented tumors from developing in six out of ten mice in the Treated Group. Only one normal immunoglobulin-treated mouse failed to develop a tumor over the course of the test. In the four anti-G_{17} treated animals that developed tumors, the mean tumor volume was reduced greater than four-fold compared to the tumors that developed in the mice treated with normal rat immunglobulin. The Results demonstrate that treatment with anti-human G_{17} immunoglobulin inhibits the development and growth of HCT-116 tumors in nude mice.

Table 5

	Treatment	
	Normal Rat Immunoglobulin	Anti-G_{17} Immunoglobulin
Mean Sera Anti-G_{17} ABC Titer (Range)	0.0 pg/μl	11.1 pg/μl (7.6-14.8)
Number of Mice Developing Tumors	9	4
Mean Tumor Volume ± S.E.	21.2 ± 11.2 mm^3	4.7 ±2.8 mm^3

EXAMPLE 9

This test demonstrates that antibody - mediated immunity to G_{17} can be selectively and safely reversed by the injection of peptide capable of binding to the antibody.

Six rats that were twice previously immunized with human gastrin hG_{17}(1-6)-DT immunogen and which exhibited anti-human gastrin antigen binding capacity off 17-34 pg/μl of serum were prepared for the standard stomach perfusion procedure. To demonstrate that each immunized rat was able to inhibit the acid secretion stimulatory activity of human G_{17}, a standard dose of approximately 120 ng of G_{17} hormone was administered to each rat. After measuring the human gastrin G_{17} stimulated acid output in response to the 120 ng of G_{17}, a second dose off human gastrin G_{17}, of 2.5 μg of hormone at twenty times the first dose was given to each rat and the gastrin stimulated acid output was measured again.

Two of the rats were then euthanized and their kidneys removed for sectioning and examination for deposition of complexes of gastrin and anti-gastrin antibody. Two of the remaining rats were then euthanized and their kidneys removed for sectioning. The remaining two rats were given 250 μg of human gastrin G_{17}, were followed for gastrin stimulated acid secretion, and then were euthanized and their kidneys were removed for sectioning.

Rapid reversal of human gastrin G_{17} neutralizing activity of the anti-sera of human gastrin G_{17} immunized rats is demonstrated in Figure 9. As expected from our previous perfusion assays, administration of approximately 120 ng of human gastrin G_{17} resulted in an 87% mean inhibition of the expected gastrin stimulated acid secretion of all the rats. Challenge with 2.5 μg off human gastrin G_{17} resulted in a gastrin-stimulated acid response identical to that seen in non-immunized rats. Challenge with 25 μg or 250 μg of human gastrin G_{17} resulted in an exaggerated acid secretion response. Kidney sections taken from these rats treated with large amounts of human gastrin G_{17} were all negative for formation and deposition of immune complexes.

Treatment of rats immunized with human gastrin G_{17} with only 2.5 μg of human gastrin G_{17} immediately reversed the neutralization of gastrin-stimulated acid secretion that was observed in these rats when they were first challenged with 120 ng of human gastrin G_{17}. Based on the antigen binding capacities of these rats, which exhibited a range of 17-34 pg/μl and a mean of 24 pg/μl, 2.5 μg of G_{17} is at least a four-fold excess of hormone injected over the total antigen binding capacity of the rat's serum. Such a small amount of hormone, if given in the same proportion based on body weight to humans would amount to a range of only 500-700 μg of human G_{17}.

Preferably the antibody neutralizing by infusion would utilize a monovalent neutralizing molecule that bears the gastrin epitope but which does not itself induce acid secretion (e.g., by changing the C-terminal end of G_{17}).

The antibody neutralizer will not prevent a renewed production of anti-gastrin antibodies, the duration of which is determined by the conditions of immunization. However, it will neutralize the antibodies as they are produced. In practice, it may be necessary in most cases to provide for neutralization of antibodies

synthesized after the initial dose of neutralizing compound is administered. This could be accomplished by means of additional infusions or, preferably, through the administration of the neutralizer in a sustained release compound or device. Although such administration would continue until the synthesis of anti-gastrin antibodies ceases, the quantity of antibody to be neutralized would be significantly less than that eliminated by the first administration of neutralizer. Consequently, the dose/frequency of neutralizer adminstrations would be diminished as antibody production subsides.

This invention and its preferred embodiments have been described in detail. It will be appreciated that those skilled in the art, upon consideration of this disclosure, may make modifications and improvements within the scope of this invention.

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Claims

Claims for the following Contracting States : AT, BE, CH, DE, DK, FR, GB, IT, LI, LU, NL, SE

1. An immunogen comprising an immunogenic carrier conjugated to a peptide residue, which comprises a fragment of the N-terminal amino acid sequence of heptadecagastrin ("G_{17}") beginning with the N-terminal amino acid thereof and ending with an amino acid thereof up to residue number 12, or a modification of such a fragment wherein the amino acid sequence differs by one or more amino acids from the natural fragment, said immunogen being capable of inducing antibodies which are specific to G_{17}.

2. An immunogen according to claim 1, wherein the peptide residue comprises the fragment coupled to a spacer peptide chain.

3. An immunogen according to claim 2, wherein the spacer peptide chain is Arg-Pro-Pro-Pro-Pro-Cys-.

4. An immunogen according to claim 1, 2 or 3, wherein the fragment comprises one of the following amino acid sequences:
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu,
pGlu-Gly-Pro-Trp-Leu,
pGlu-Gly-Pro-Trp,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu.

5. An immunogen according to claims 1, 2 or 3, wherein the fragment comprises the amino acid sequence:
pGlu-Gly-Pro-Trp-Leu-Glu or
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu.

6. An immunogen according to claims 1, 2 or 3, wherein the peptide residue is selected from the following:
pGlu-Gly-Pro-Trp-Leu-Glu-Arg-Pro-Pro-Pro-Pro-Cys-.
pGlu-Gly-Pro-Trp-Leu-Arg-Pro-Pro-Pro-Pro-Cys-.
pGlu-Gly-Pro-Trp-Arg-Pro-Pro-Pro-Pro-Cys-.
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Lys-Arg-Pro-Pro-Pro-Pro-Lys-.

7. An immunogen comprising a peptide selected from:
p-Glu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu,
p-Glu-Gly-Pro-Trp-Leu-Glu-Glu-Glu,
p-Glu-Gly-Pro-Trp-Leu-Glu-Glu,
p-Glu-Gly-Pro-Trp-Leu-Glu,
p-Glu-Gly-Pro-Trp-Leu and
p-Glu-Gly-Pro-Trp,
coupled to an immunogenic carrier.

8. An immunogen according to any preceding claim, wherein the immunogenic carrier is selected from: diphtheria toxoid, tetanus toxoid, keyhole limpet hemocyanin, bovine serum albumin and fragments thereof.

9. An immunogen comprising a number of peptide residues as defined in claim 1 bound on their C-terminal ends to a polymer consisting of crosslinked peptide units.

10. An immunogen according to claim 9, wherein the peptide residues are bound to an amino acid sequence having free amino groups which are crosslinked with glutaraldehyde.

11. A pharmaceutical composition comprising an immunogen defined in any preceding claim and a pharmaceutically acceptable carrier and/or adjuvant.

12. An immunogen according to any one of claims 1-10, for use in the control or regulation of:
(i) a gastrin-induced disorder,
(ii) another disease which appears to be related to a hormonal or stimulatory effect of gastrin, or
(iii) a condition in which gastrin is overproduced.

13. An immunogen according to any one of claims 1-10, for use in the treatment of cancers of the gastro-intestinal tract,

14. An immunogen according to any one of claims 1-10, for use in the treatment of peptic ulcers.

15. Antibodies against gastrin hormone which selectively bind with heptadecagastrin (G_{17}), for use in a treatment defined in claim 12, 13 or 14.

16. Antibodies according to claim 15, which are generated from an immunogen as defined in any one of claims 1-10.

17. A pharamaceutical composition comprising antibodies defined in claim 15 or 16 and a pharmaceutically acceptable carrier and/or adjuvant.

18. A peptide as defined in claim 1, for use in reversing immunity to G_{17} mediated by antibodies defined in claim 15 or 16.

19. A peptide comprising a heptadecagastrin (G_{17}) fragment consisting of the sequence
p-Glu-Gly-Pro-Trp-Leu-Glu-Glu-Glu,
p-Glu-Gly-Pro-Trp-Leu-Glu-Glu,
p-Glu-Gly-Pro-Trp-Leu-Glu,
p-Glu-Gly-Pro-Trp-Leu or
p-Glu-Gly-Pro-Trp.

20. A peptide comprising a heptadecagastrin (G_{17}) fragment consisting of the sequence
p-Glu-Gly-Pro-Trp-Leu-Glu or
p-Glu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu.

21. Use in the preparation of an immunogen defined in claim 1 of a peptide according to claim 19 or 20.

22. A peptide claimed in claim 19 or 20, for use in reversing immunity to G_{17} mediated by antibodies defined in claim 15.

23. Use of an immunogen according to any one of claims 1-10 for the preparation of a pharmaceutical composition for use in the control or regulation of:
(i) a gastrin-induced disorder,
(ii) another disease which appears to be related to a hormonal or stimulatory effect of gastrin, or
(iii) a condition in which gastrin is overproduced.

24. Use of an immunogen according to any one of claims 1-10 for the preparation of a pharmaceutical composition for use in the treatment of cancers of the gastro-intestinal tract.

25. Use of an immunogen according to any one of claims 1-10 for the preparation of a pharmaceutical composition for use in the treatment of peptic ulcers.

Claims for the following Contracting States : ES, GR

1. A process for the preparation of an immunogen comprising an immunogenic carrier conjugated to a peptide residue, which comprises a fragment of the N-terminal amino acid sequence of heptadecagastrin ("G_{17}") beginning with the N-terminal amino acid thereof and ending with an amino acid thereof up to residue number 12, or a modification of such a fragment wherein the amino acid sequence differs by one or more amino acids from the natural fragment, said immunogen being capable of inducing antibodies which are specific to G_{17}, said process being characterised in that one synthesises a said peptide from its constituent amino acids and conjugates it to the immunogenic carrier.

2. A process according to claim 1, characterised in that one synthesises a peptide residue comprising the fragment coupled to a spacer peptide chain.

3. A process according to claim 2, characterised in that one synthesises a the spacer peptide chain Arg-Pro-Pro-Pro-Pro-Cys-.

4. A process according to claim 1, 2 or 3, characterised in that one synthesises a fragment comprising one of the following amino acid sequences:
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu,
pGlu-Gly-Pro-Trp-Leu,
pGlu-Gly-Pro-Trp,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu.

5. A process according to claims 1, 2 or 3, characterised in that one synthesises a fragment comprising the amino acid sequence:
pGlu-Gly-Pro-Trp-Leu-Glu or
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu.

6. A process according to claims 1, 2 or 3, characterised in that one synthesises a peptide residue selected from the following:
pGlu-Gly-Pro-Trp-Leu-Glu-Arg-Pro-Pro-Pro-Pro-Cys-.
pGlu-Gly-Pro-Trp-Leu-Arg-Pro-Pro-Pro-Pro-Cys-.
pGlu-Gly-Pro-Trp-Arg-Pro-Pro-Pro-Pro-Cys-.
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Lys-Arg-Pro-Pro-Pro-Pro-Lys-.

7. A process according to claim 1, characterised in that one synthesises a peptide selected from:
p-Glu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu,
p-Glu-Gly-Pro-Trp-Leu-Glu-Glu-Glu,
p-Glu-Gly-Pro-Trp-Leu-Glu-Glu,
p-Glu-Gly-Pro-Trp-Leu-Glu,
p-Glu-Gly-Pro-Trp-Leu and
p-Glu-Gly-Pro-Trp,
and conjugates it to an immunogenic carrier.

8. A process according to any preceding claim, characterised in that one conjugates the peptide to an immunogenic carrier selected from: diphtheria toxoid, tetanus toxoid, keyhole limpet hemocyanin, bovine serum albumin and fragments thereof.

9. A process of preparing an immunogen, characterised in that one synthesises (i) a number of peptide residues as defined in claim 1 and (ii) further synthesises other, crosslinkable, peptide units, which one joins to the C-terminal ends of said peptide residues and crosslinks.

10. A process according to claim 9, characterised in that one synthesises said other peptide units having free amino groups which are crosslinked with glutaraldehyde.

11. A process according to any preceding claim, characterised in that one synthesises the peptide by a known method of solid state synthesis.

12. A process according to any preceding claim, characterised in that one formulates the immunogen with a pharmaceutically acceptable carrier and/or adjuvant.

13. A process according to claim 12, characterised in that one formulates the immunogen for use in the control or regulation of:
(i) a gastrin-induced disorder,
(ii) another disease which appears to be related to a hormonal or stimulatory effect of gastrin, or
(iii) a condition in which gastrin is overproduced.

14. A process according to claim 13, characterised in that one formulates the immunogen, for use in the treatment of cancers of the gastro-intestinal tract,

15. A process according to claim 13, characterised in that one formulates the immunogen for use in the treatment of peptic ulcers.

16. A process for the preparation of antibodies which selectively bind with heptadecagastrin (G_{17}), for use in a treatment defined in claim 13, 14 or 15, characterised in that one raises them against an immunogen as defined in any one of claims 1-11.

Patentansprüche
Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, DE, DK, FR, GB, IT, LI, LU, NL, SE

1. Immunogen, einen immunisierenden Träger einschließend, konjugiert zu einem Peptidrest, der ein Fragment der N-terminalen Aminosäurensequenz von Heptadecagastrin ("G_{17}"), beginnend mit der N-terminalen Aminosäure davon und endend mit einer Aminosäure davon bis zum Rest Nummer 12, oder eine Modifikation eines solchen Fragments, in welchem die Aminosäurensequenz sich durch eine oder mehrere Aminosäuren von dem natürlichen Fragment unterscheidet, umfaßt, wobei das Immunogen in der Lage ist, Antikörper herbeizuführen, die spezifisch zu G_{17} sind.

2. Immunogen nach Anspruch 1, in welchem der Peptidrest das Fragment umfaßt, welches an eine Spacer Peptidkette gekoppelt ist.

3. Immunogen nach Anspruch 2, in dem die Spacer Peptidkette Arg-Pro-Pro-Pro-Pro-Cys ist.

4. Immunogen nach Anspruch 1, 2 oder 3, in dem das Fragment eine der folgenden Aminosäurensequenzen umfaßt:
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu,
pGlu-Gly-Pro-Trp-Leu,
pGlu-Gly-Pro-Trp,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu.

5. Immunogen nach den Ansprüchen 1, 2 oder 3, in dem das Fragment die Aminosäurensequenz
pGlu-Gly-Pro-Trp-Leu-Glu oder
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu umfaßt.

6. Immunogen nach Anspruch 1 , 2 oder 3, in dem der Peptidrest von den folgenden ausgewählt ist:
pGlu-Gly-Pro-Trp-Leu-Glu-Arg-Pro-Pro-Pro-Pro-Cys.
pGlu-Gly-Pro-Trp-Leu-Arg-Pro-Pro-Pro-Pro-Cys.
pGlu-Gly-Pro-Trp-Arg-Pro-Pro-Pro-Pro-Cys.
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Lys-Arg-Pro-Pro-Pro-Pro-Lys.

7. Immunogen, welches ein Peptid enthält, das ausgewählt ist von:
p-Glu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu,
p-Glu-Gly-Pro-Trp-Leu-Glu-Glu-Glu,
p-Glu-Gly-Pro-Trp-Leu-Glu-Glu,
p-Glu-Gly-Pro-Trp-Leu-Glu,
p-Glu-Gly-Pro-Trp-Leu und
p-Glu-Gly-Pro-Trp.

8. Immunogen nach einem der vorhergehenden Ansprüche, in welchem der immunisierende Träger ausgewählt ist von: Diphterietoxoid, Tetanustoxoid, keyhole limpet Hämocyanin, Rinderserumalbumin und Fragmente davon.

9. Immunogen, eine Anzahl von Peptidresten umfassend, gemäß Anspruch 1, die an ihre C-Terminalenden zu einem Polymer gebunden sind, bestehend aus vernetzten Peptideinheiten.

10. Immunogen nach Anspruch 9, in welchem die Peptidreste an eine Aminosäurensequenz mit freien Aminogruppen gebunden sind, die mit Glutaraldehyd vernetzt sind.

11. Pharmazeutische Zusammensetzung mit einem Immunogen nach einem der vorhergehenden Ansprüche und einem pharmazeutisch akzeptablem Träger und/oder Adjuvans.

12. Immunogen nach einem der Ansprüche 1 bis 10 zur Verwendung bei der Steuerung oder Reglung von
(i) einer durch Gastrin herbeigeführten Störung,
(ii) einer anderen Krankheit, welche mit einem hormonalen oder stimulierenden Effekt von Gastrin verwandt zu sein scheint, oder
(iii) eines Zustandes, in welchem Gastrin überproduziert wird.

13. Immunogen nach einem der Ansprüche 1 bis 10 zur Verwendung bei der Behandlung von Krebs im Magen-Darm-Trakt.

14. Immunogen nach einem der Ansprüche 1 bis 10 zur Verwendung bei der Behandlung von Magengeschwüren.

15. Antikörper gegen Gastrinhormone, welche sich selektiv mit Heptadecagastrin (G_{17}) binden, zur Verwendung bei Behandlungen nach den Ansprüchen 12, 13 oder 14.

16. Antikörper nach Anspruch 15, welche von einem Immunogen nach einem der Ansprüche 1 bis 10 erzeugt wurden.

17. Pharmazeutische Zusammensetzung mit Antikörpern nach Anspruch 15 oder 16 und ein pharmazeutisch akzeptabler Träger und/oder Adjuvans.

18. Peptid nach Anspruch 1, zur Verwendung bei der Umkehrimmunität zu G_{17}, vermittelt durch Antikörper, nach Anspruch 15 oder 16.

19. Peptid mit einem Heptadecagastrin (G_{17}) Fragment, bestehend aus der Sequenz
p-Glu-Gly-Pro-Trp-Leu-Glu-Glu-Glu,
p-Glu-Gly-Pro-Trp-Leu-Glu-Glu,
p-Glu-Gly-Pro-Trp-Leu-Glu,
p-Glu-Gly-Pro-Trp-Leu oder
p-Glu-Gly-Pro-Trp.

20. Peptid mit einem Heptadecagastrin (G_{17}) Fragment, bestehend aus der Sequenz
p-Glu-Gly-Pro-Trp-Leu-Glu oder
p-Glu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu.

21. Anwendung bei der Vorbereitung eines Immunogens nach Anspruch 1 eines Peptids nach Anspruch 19 oder 20.

22. Peptid nach Anspruch 19 oder 20 zur Anwendung in der Umkehrimmunität zu G_{17}, vermittelt durch Antikörper nach Anspruch 15.

23. Anwendung eines Immunogens nach einem der Ansprüche 1 bis 10 zur Vorbereitung einer pharmazeutischen Zusammensetzung zur Anwendung bei der Steuerung oder Regelung
(i) eines durch Gastrin erzeugten Leidens,
(ii) einer anderen Krankheit, welche mit einer hormonellen oder Stimulierwirkung von Gastrin verwandt zu sein scheint, oder
(iii) eines Leidens, bei welchem Gastrin überproduziert ist.

24. Anwendung eines Immunogens nach einem der Ansprüche 1 bis 10 für die Vorbereitung einer pharmazeutischen Zusammensetzung zur Verwendung bei der Behandlung von Krebs des Magen-Darm-Trakts.

25. Anwendung eines Immunogens nach einem der Ansprüche 1 bis 10 für die Vorbereitung einer pharmazeutischen Zusammensetzung zur Verwendung bei der Behandlung von Magengeschwüren.

Patentansprüche für folgende Vertragsstaaten : ES, GR

1. Verfahren zur Herstellung eines Immunogens, einen immunisierenden Träger einschließend, konjugiert zu einem Peptidrest, der ein Fragment der N-terminalen Aminosäurensequenz von Heptadecagastrin ("G_{17}"), beginnend mit der N-terminalen Aminosäure davon und endend mit einer Aminosäure davon bis zum Rest Nummer 12, oder eine Modifikation eines solchen Fragments, in welchem die Aminosäurensequenz sich durch eine oder mehrere Aminosäuren von dem natürlichen Fragment unterscheidet, umfaßt, wobei das Immunogen in der Lage ist, Antikörper herbeizuführen, die spezifisch zu G_{17} sind, dadurch gekennzeichnet, daß man das Peptid von seinem Aminosäurenkonstituent synthetisiert und zu dem immunisierenden Träger konjugiert.

2. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß man einen Peptidrest synthetisiert, der das Fragment umfaßt, welches an eine Spacer-Peptidkette gekoppelt ist.

3. Verfahren nach Anspruch 2, dadurch gekennzeichnet, daß man die Spacer-Peptidkette Arg-Pro-Pro-Pro-Pro-Cys synthetisiert.

4. Verfahren nach Anspruch 1, 2 oder 3, dadurch gekennzeichnet, daß man ein Fragment synthetisiert, daß eine der folgenden Aminosäurensequenzen umfaßt:
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu,
pGlu-Gly-Pro-Trp-Leu,
pGlu-Gly-Pro-Trp,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu.

5. Verfahren nach Anspruch 1, 2 oder 3, dadurch gekennzeichnet, daß man ein Fragment synthetisiert, daß die Aminosäurensequenz
pGlu-Gly-Pro-Trp-Leu-Glu oder
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu umfaßt.

6. Verfahren nach Anspruch 1, 2 oder 3, dadurch gekennzeichnet, daß man einen Peptidrest synthetisiert, ausgewählt aus folgenden:

pGlu-Gly-Pro-Trp-Leu-Glu-Arg-Pro-Pro-Pro-Pro-Cys-.
pGlu-Gly-Pro-Trp-Leu-Arg-Pro-Pro-Pro-Pro-Cys-.
pGlu-Gly-Pro-Trp-Arg-Pro-Pro-Pro-Pro-Cys-.
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Lys-Arg-Pro-Pro-Pro-Pro-Lys-.

7. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß man ein Peptidrest synthetisiert, ausgewählt aus:
p-Glu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu,
p-Glu-Gly-Pro-Trp-Leu-Glu-Glu-Glu,
p-Glu-Gly-Pro-Trp-Leu-Glu-Glu,
p-Glu-Gly-Pro-Trp-Leu-Glu,
p-Glu-Gly-Pro-Trp-Leu und
p-Glu-Gly-Pro-Trp,
und konjugiert zu einem immunisierenden Träger.

8. Verfahren nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß man das Peptid zu einem immunisierenden Träger konjugiert, ausgewählt von: Diphterietoxoid, Tetanustoxoid, keyhole limpet Hämocyanin, Rinderserumalbumin und Fragmenten davon.

9. Verfahren zur Herstellung eines Immunogens, dadurch gekennzeichnet, daß man (i) eine Anzahl von Peptidresten gemäß Anspruch 1 und (ii) weiterhin andere vernetzbare Peptideinheiten synthetisiert, welche man mit den C-terminalen Enden der genannten Peptidreste verbindet und vernetzt.

10. Verfahren nach Anspruch 9, dadurch gekennzeichnet, daß man die genannten anderen Peptideinheiten, die freie Aminogruppen haben, welche mit Glutaraldehyd vernetzt sind, synthetisiert.

11. Verfahren nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß das Peptid durch ein bekanntes Verfahren der Festkörpersynthese synthetisiert wird.

12. Verfahren nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß man das Immunogen mit einem pharmazeutisch akzeptablen Träger und/oder Adjuvans formuliert.

13. Verfahren nach Anspruch 12, dadurch gekennzeichnet, daß man das Immunogen zur Verwendung bei der Steuerung oder Regelung
(i) einer durch Gastrin herbeigeführten Störung,
(ii) einer anderen Krankheit, welche mit einem hormonalen oder stimulierenden Effekt von Gastrin verwandt zu sein scheint, oder
(iii) eines Zustandes, in welchem Gastrin überproduziert wird, formuliert.

14. Verfahren nach Anspruch 13, dadurch gekennzeichnet, daß man das Immunogen zur Verwendung bei der Behandlung von Krebs im Magen-Darm-Trakt formuliert.

15. Verfahren nach Anspruch 13, dadurch gekennzeichnet, daß man das Immunogen zur Verwendung bei der Behandlung von Magengeschwüren formuliert.

16. Verfahren zur Herstellung von Antikörpern, welche sich selektiv mit Heptadecagastrin (G_{17}) binden, zur Verwendung bei der Behandlung nach Anspruch 13, 14 oder 15, dadurch gekennzeichnet, daß man sie gegen ein Immunogen nach den Ansprüchen 1 bis 11 einsetzt.

Revendications

Revendications pour les Etats contractants suivants : AT, BE, CH, DE, DK, FR, GB, IT, LI, LU, NL, SE

1. Immunogène comprenant un porteur immunogène conjugué à un résidu peptidique, qui comporte un fragment de la séquence d'acides aminés N-terminale de l'heptadécagastrine ("G_{17}") qui commence par l'acide aminé N-terminal et se prolonge jusqu'à l'acide aminé correspondant au résidu numéro 12, ou une variante d'un tel fragment dans laquelle la séquence d'acides aminés diffère du fragment natif par un ou plusieurs acides aminés, ledit immunogène étant capable d'induire la production d'anticorps spécifiques de la G_{17}.

2. Immunogène selon la revendication 1, dans lequel le résidu peptidique comprend le fragment couplé à une chaîne peptidique intercalaire.

3. Immunogène selon la revendication 2, dans la chaîne peptidique intercalaire est Arg-Pro-Pro-Pro-Pro-Cys-.

4. Immunogène selon la revendication 1, 2 ou 3, dans lequel le fragment comprend l'une des séquences d'acides aminés suivantes:
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu,
pGlu-Gly-Pro-Trp-Leu,
pGlu-Gly-Pro-Trp,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu.

5. Immunogène selon la revendication 1, 2 ou 3 dans lequel le fragment comprend la séquence d'acides aminés:
pGlu-Gly-Pro-Trp-Leu-Glu ou
pGlu-Glu-Pro-Trp-Leu-Glu-Glu-Glu-Glu.

6. Immunogène selon la revendication 1, 2 ou 3 dans lequel le résidu peptidique est choisi parmi ce qui suit:
pGlu-Gly-Pro-Trp-Leu-Glu-Arg-Pro-Pro-Pro-Pro-Cys-.
pGlu-Gly-Pro-Trp-Leu-Arg-Pro-Pro-Pro-Pro-Cys-.
pGlu-Gly-Pro-Trp-Arg-Pro-Pro-Pro-Pro-Cys-.
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Lys-Arg-Pro-Pro-Pro-Pro-Lys-.

7. Immunogène comprenant un peptide choisi parmi :
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu,
pGlu-Gly-Pro-Trp-Leu-Glu,
pGlu-Gly-Pro-Trp-Leu et
pGlu-Gly-Pro-Trp-,
couplés à un immunogène porteur.

8. Immunogène selon l'une quelconque des revendications précédentes dans lequel le porteur immunogène est choisi parmi : l'anatoxine diphtérique, l'anatoxine tétanique, l'hémocyanine de *Megathura crenulata* (KLH), la sérumalbumine bovine et les fragments de ces derniers.

9. Immunogène comprenant plusieurs résidus peptidiques, tels que définis dans la revendication 1, liés sur leurs extrémités C-terminales à un polymère constitué de motifs peptidiques réticulés.

10. Immunogène selon la revendication 9, dans lequel les résidus peptidiques sont liés à une séquence d'acides aminés présentant des groupes amino libres qui subissent une réticulation en présence de glutaraldéhyde.

11. Composition pharmaceutique comprenant un immunogène défini dans l'une quelconque des revendications précédentes en association avec un véhicule et/ou un adjuvant pharmaceutiquement acceptables.

12. Immunogène selon l'une quelconque des revendications 1 à 10 pouvant servir à contrôler ou réguler
(i) un trouble induit par la gastrine,
(ii) une autre maladie qui semble être liée à l'effet hormonal ou stimulateur de la gastrine, ou
(iii) un état caractérisé par une surproduction de gastrine.

13. Immunogène selon l'une quelconque des revendications 1 à 10, destiné à être utilisé dans le traitement des cancers du tube gastro-intestinal.

14. Immunogène selon l'une quelconque des revendications 1 à 10, destiné à être utilisé dans le traitement des ulcères de l'estomac.

15. Anticorps dirigés contre l'hormone gastrine qui se lient de manière sélective à l'heptadécagastrine (G17) destinés à une utilisation thérapeutique telle que
définie dans la revendication 12, 13 ou 14.

16. Anticorps selon la revendication 15, qui sont induits par un immunogène tel que défini dans l'une quelconque des revendications 1 à 10.

17. Composition pharmaceutique comprenant des anticorps tel que définis dans la revendication 15 ou 16 et un véhicule et/ou un adjuvant pharmaceutiquement acceptables.

18. Peptide tel que défini dans la revendication 1, pouvant servir à moduler vers le bas l'immunité envers G_{17} qui est médiée par des anticorps tels que définis dans la revendication 15 ou 16.

19. Peptide comprenant un fragment de l'heptadécagastrine (G_{17}) constitué de la séquence
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu,
pGlu-Gly-Pro-Trp-Leu-Glu,
pGlu-Gly-Pro-Trp-Leu ou
pGlu-Gly-Pro-Trp.

20. Peptide comprenant un fragment de l'heptadécagastrine (G_{17}) constitué de la séquence
p-Glu-Gly-Pro-Trp-Leu-Glu ou
p-Glu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu.

21. Utilisation d'un peptide selon la revendication 19 ou 20 en vue de préparer un immunogène défini dans la revendication 1

22. Peptide tel que revendiqué dans la revendication 19 ou 20, servant à moduler vers le bas l'immunité envers G_{17} qui est médiée par des anticorps tels que définis dans la revendication 15.

23. Utilisation d'un immunogène selon l'une quelconque des revendications 1 à 10 en vue de préparer une composition pharmaceutique pouvant servir à contrôler ou réguler
(i) un trouble induit par la gastrine
(ii) une autre maladie qui semble être liée à l'effet hormonal ou stimulateur de la gastrine, ou
(iii) un état caractérisé par une surproduction de gastrine.

24. Utilisation d'un immunogène selon l'une quelconque des revendications 1 à 10, en vue de préparer une composition pharmaceutique destinée à être employée dans le traitement des cancers du tube gastro-intestinal.

25. Utilisation d'un immunogène selon l'une quelconque des revendications 1 à 10, en vue de préparer une composition pharmaceutique destinée à être employée dans le traitement des ulcères de l'estomac.

Revendications pour les Etats contractants suivants : ES, GR

1. Procédé de préparation d'un immunogène comprenant un porteur immunogène conjugué à un résidu peptidique, qui comporte un fragment de la séquence d'acides aminés N-terminale de l'heptadécagastrine ("G_{17}") qui commence par l'acide aminé N-terminal et se prolonge jusqu'à l'acide aminé correspondant au résidu numéro 12, ou une variante d'un tel fragment dans laquelle la séquence d'acides aminés diffère du fragment natif par un ou plusieurs acides aminés, ledit immunogène étant capable d'induire la production d'anticorps spécifiques de la G_{17}, ledit procédé étant caractérisé en ce que l'on synthétise ledit peptide à partir des acides aminés constitutifs et en ce qu'on conjugue le peptide au porteur immunogène.

2. Procédé selon la revendication 1, caractérisé en ce que l'on synthétise un résidu peptidique comprenant le fragment couplé à une chaîne peptidique intercalaire.

3. Procédé selon la revendication 2, caractérisé en ce que l'on synthétise la chaîne peptidique intercalaire Arg-Pro-Pro-Pro-Pro-Cys-.

4. Procédé selon la revendication 1, 2 ou 3, caractérisé en ce que l'on synthétise un fragment comprenant l'une des séquences d'acides aminés suivantes:
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu,
pGlu-Gly-Pro-Trp-Leu,
pGlu-Gly-Pro-Trp,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu.

5. Procédé selon la revendication 1, 2 ou 3 caractérisé en ce que l'on synthétise un fragment comprenant la séquence d' acides aminés:
pGlu-Gly-Pro-Trp-Leu-Glu ou
pGlu-Glu-Pro-Trp-Leu-Glu-Glu-Glu-Glu.

6. Procédé selon la revendication 1, 2 ou 3 caractérisé en ce que l'on synthétise un résidu peptidique choisi parmi ce qui suit:
pGlu-Gly-Pro-Trp-Leu-Glu-Arg-Pro-Pro-Pro-Pro-Cys-.
pGlu-Gly-Pro-Trp-Leu-Arg-Pro-Pro-Pro-Pro-Cys-.
pGlu-Gly-Pro-Trp-Arg-Pro-Pro-Pro-Pro-Cys-.
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Lys-Arg-Pro-Pro-Pro-Pro-Lys-.

7. Procédé selon la revendication 1, caractérisé en ce que l'on synthétise un peptide choisi parmi :
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu,
pGlu-Gly-Pro-Trp-Leu-Glu-Glu,
pGlu-Gly-Pro-Trp-Leu-Glu,
pGlu-Gly-Pro-Trp-Leu et
pGlu-Gly-Pro-Trp-,
et qu'on le couple à un véhicule immunogène.

8. Procédé selon l'une quelconque des revendications précédentes, caractérisé en ce que l'on conjuge le peptide à un véhicule immunogène choisi parmi : l'anatoxine diphtérique, l'anatoxine tétanique, l'hémocyanine de *Mégathura crenulata* (KLH), la sérumalbumine bovine et les fragments de ces derniers.

9. Procédé de préparation d'un immunogène, caractérisé en ce que l'on synthétise (i) plusieurs résidus peptidiques tels que définis dans la revendication 1 et qu'on synthétise en outre (ii) d'autres motifs peptidiques réticulables pour les lier aux extrémités C-terminales desdits résidus peptidiques et provoquer leur réticulation.

10. Procédé selon la revendication 9, caractérisé en ce que l'on synthétise lesdits autres motifs peptidiques portant des groupes amino libres qui subissent une réticulation en présence de glutaraldéhyde.

11. Procédé selon l'une quelconque des revendications précédentes, caractérisé en ce que l'on synthétise le peptide par une technique connue de synthèse en phase solide.

12. Procédé selon l'une quelconque des revendications précédentes caractérisé en ce que l'on formule l'immunogène avec un véhicule et/ou un adjuvant pharmaceutiquement acceptables.

13. Procédé selon la revendication 12, caractérisé en ce que l'on formule l'immunogène en vue de l'utiliser dans le contrôle ou la régulation de :

(i) un trouble induit par la gastrine,
(ii) une autre maladie qui semble être liée à l'effet hormonal ou stimulateur de la gastrine, ou
(iii) un état caractérisé par une surproduction de gastrine.

14. Procédé selon la revendication 13, caractérisé en ce que l'on formule l'immunogène en vue de l'utiliser dans le traitement des cancers du tube gastro-intestinal.

15. Procédé selon la revendication 13, caractérisé en ce que l'on formule l'immunogène en vue de l'utiliser dans le traitement des ulcères de l'estomac.

16. Procédé de préparation d'anticorps qui se lient de manière sélective à l'heptadécagastrine (G_{17}), destinés à une utilisation thérapeutique telle que définie dans la revendication 13, 14 ou 15, caractérisé en ce que l'on génère ces anticorps contre un immunogène tel que défini dans l'une quelconque des revendications 1 à 11.

FIGURE 1

Figure 1 G17 with Control Rat Serum

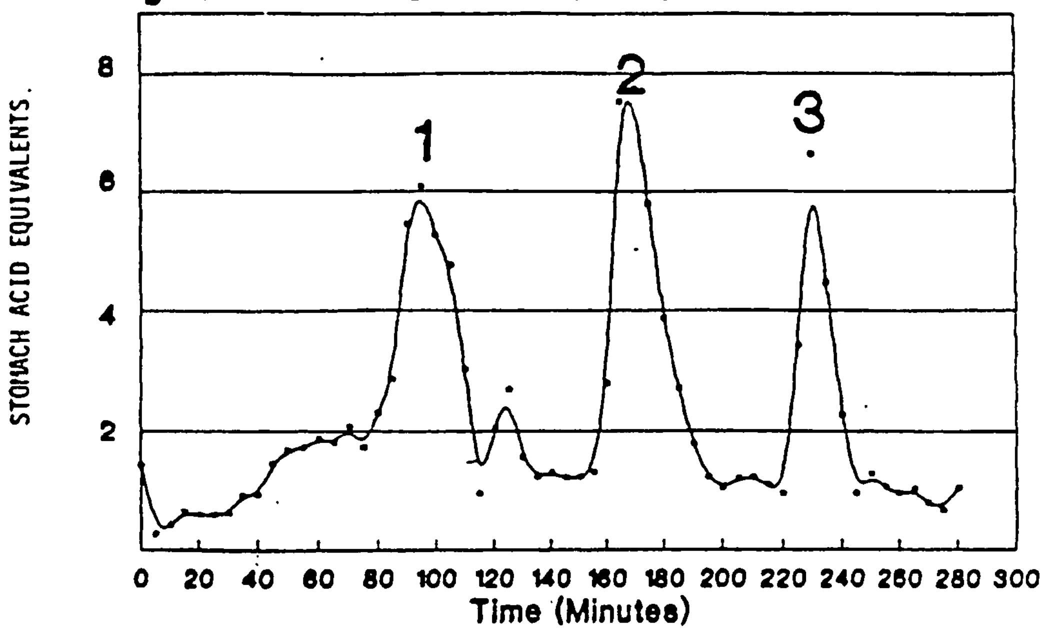


Figure 2 G17 with Rat anti-G17 Serum

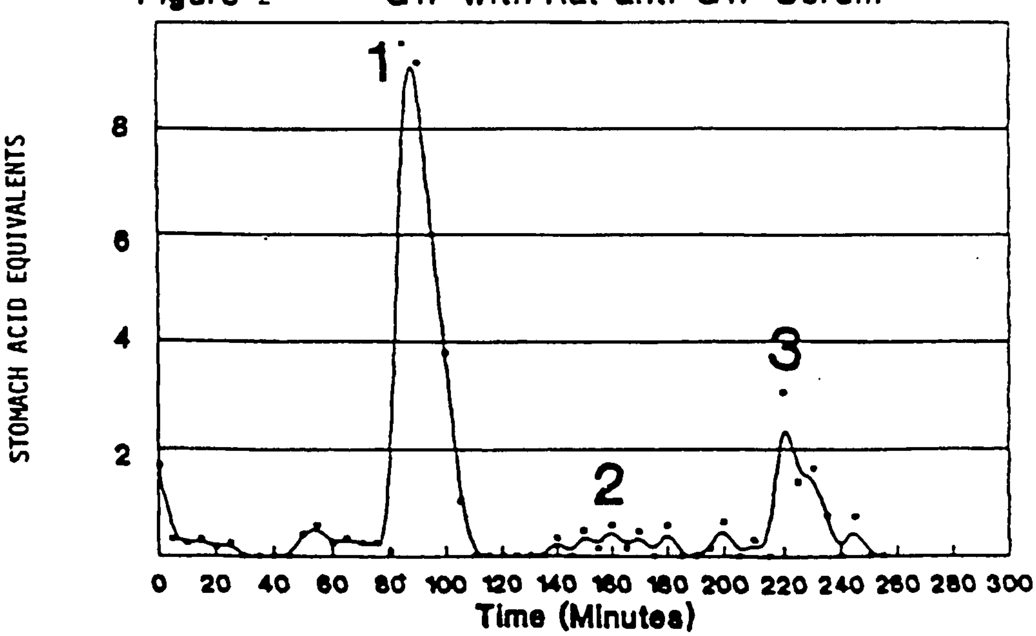
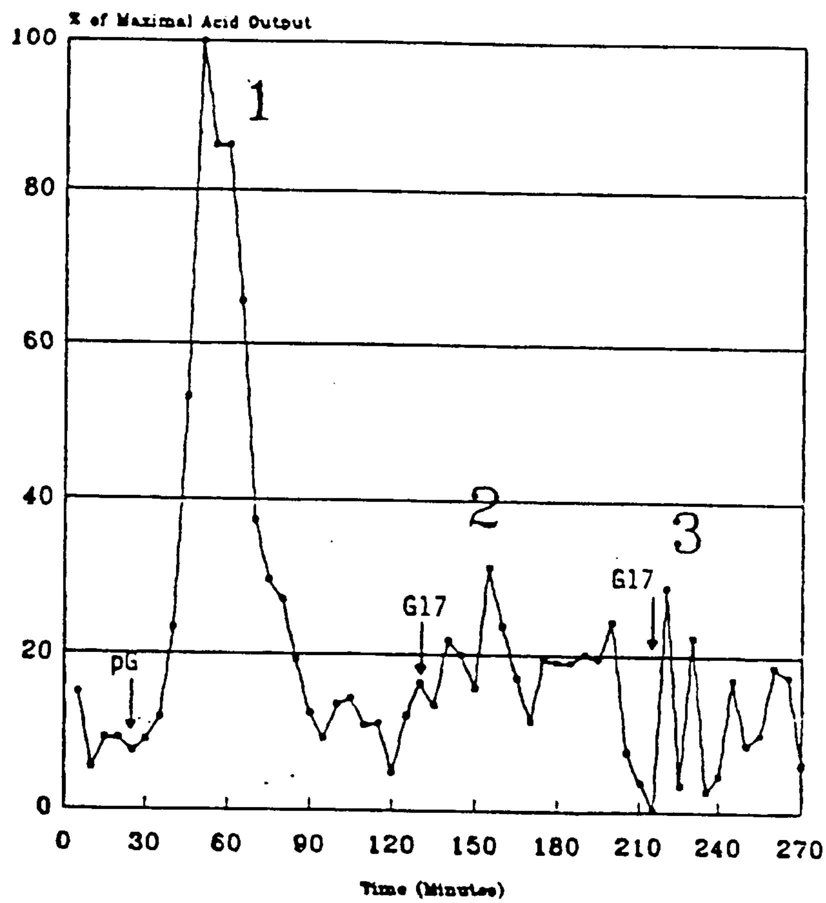


Figure 3

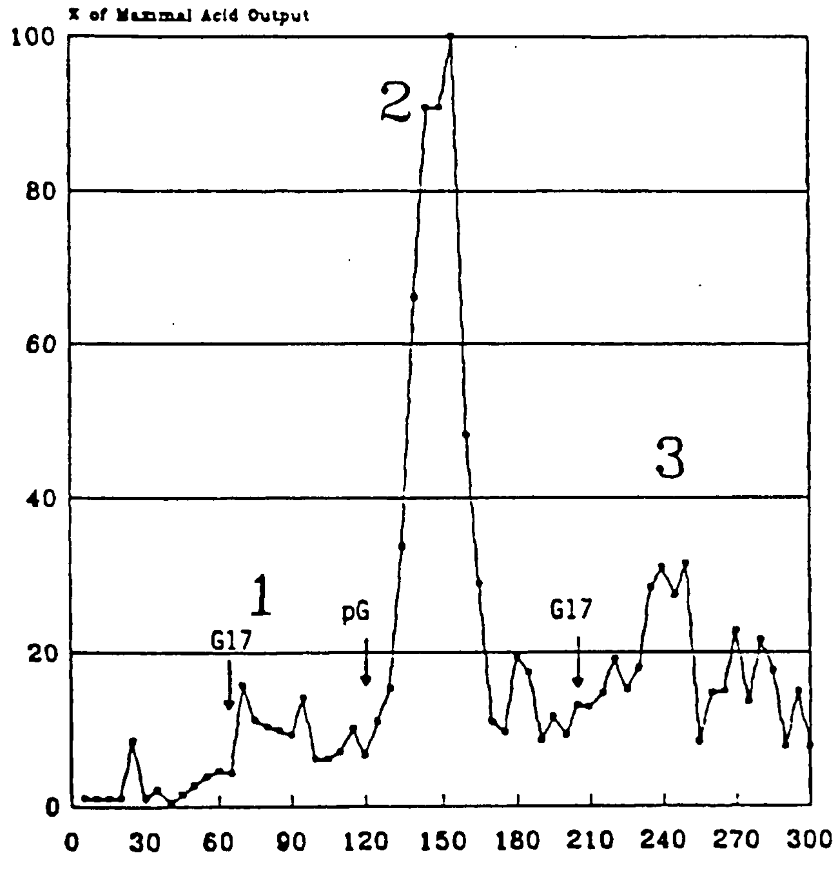
Stomach Acid Output in Response to Injection of G17 and Pentagastrin (pG) in Rat Actively Immunized Against G17



Arrows: Time of G17 or pG injection.
Acid Secretion peaks are numbered 1,2,3.

Figure 4

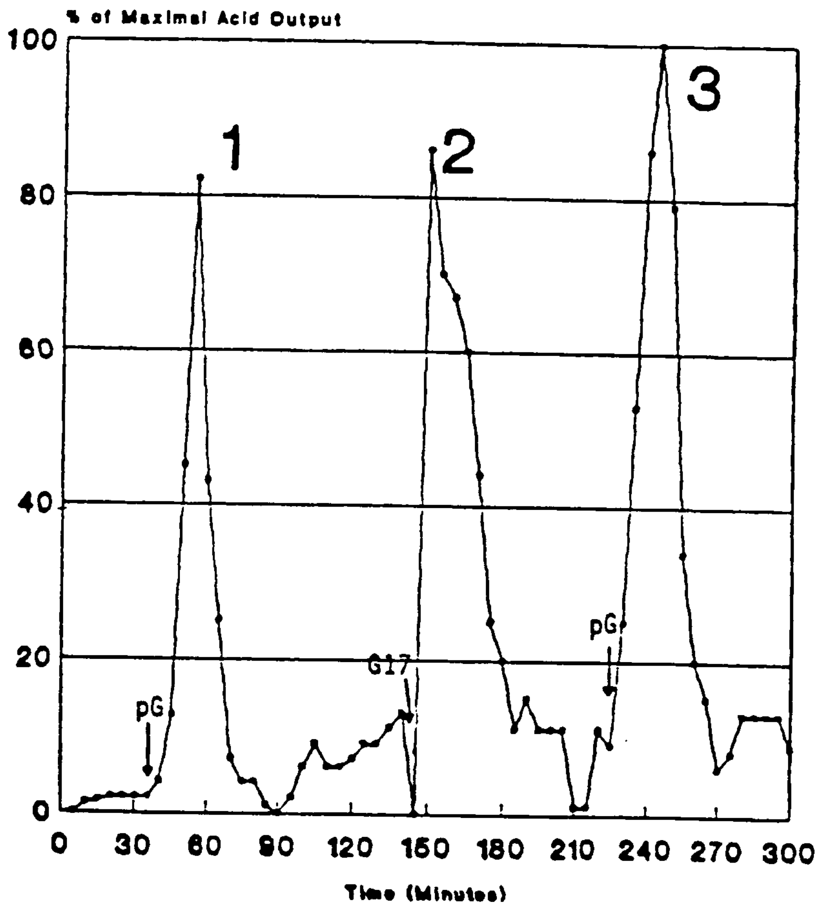
Stomach Acid Output in Response to Injection of G17 and Pentagastrin (pG) in Rat Actively Immunized Against G17



Arrows: Time of G17 or pG Injection.
Acid secretion peaks are numbered 1,2,3.

Figure 5

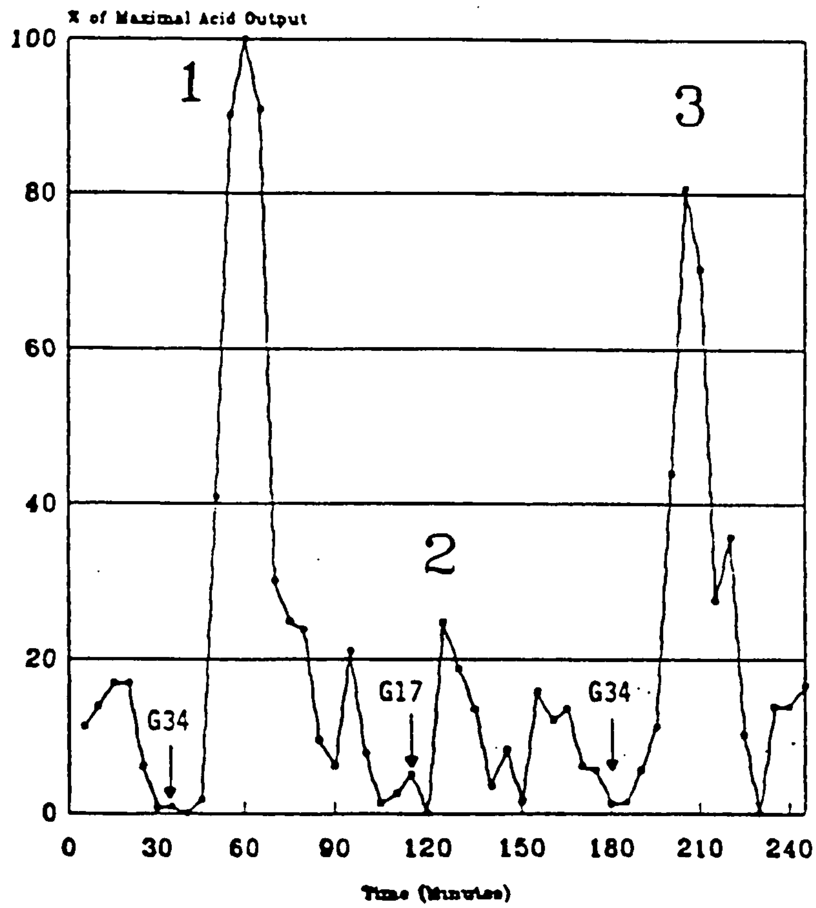
Stomach Acid Output in Response to Injection of G17 and Pentagastrin (pG) in Control Rat



Arrows: Time of G17 or pG injection.
Acid secretion peaks are numbered 1,2,3.

Figure 6

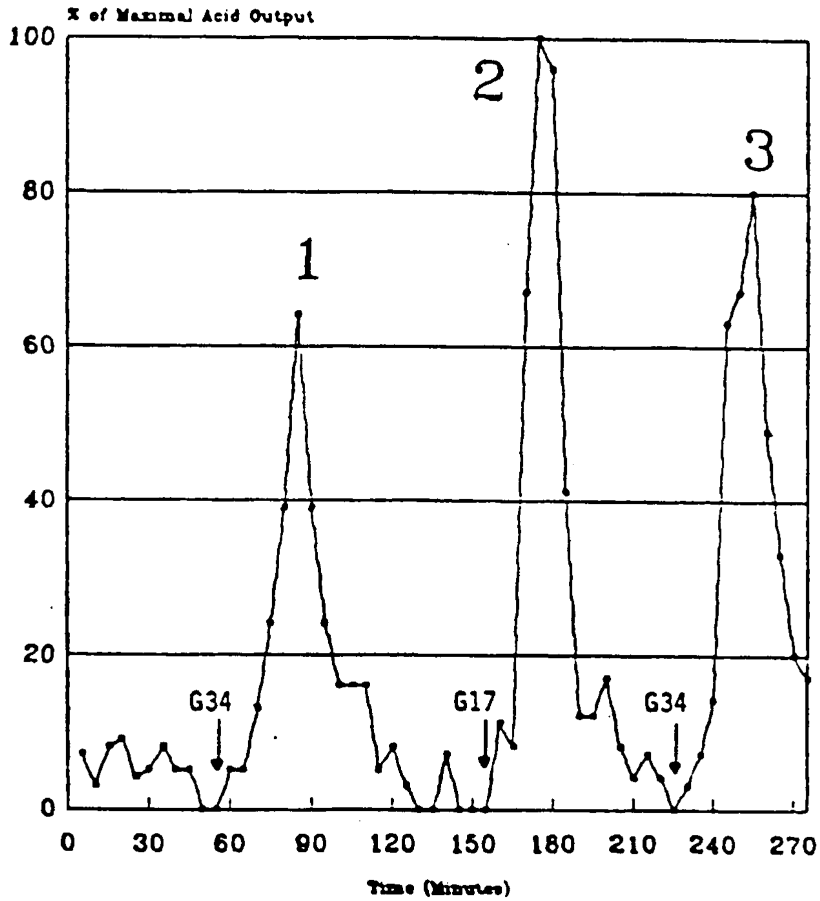
Stomach Acid Output in Response to
Injection of G17 and G34
in Rat Actively Immunized Against G17



Arrows: Time of G17 or G34 injection.
Acid Secretion peaks are numbered 1,2,3.

Figure 7

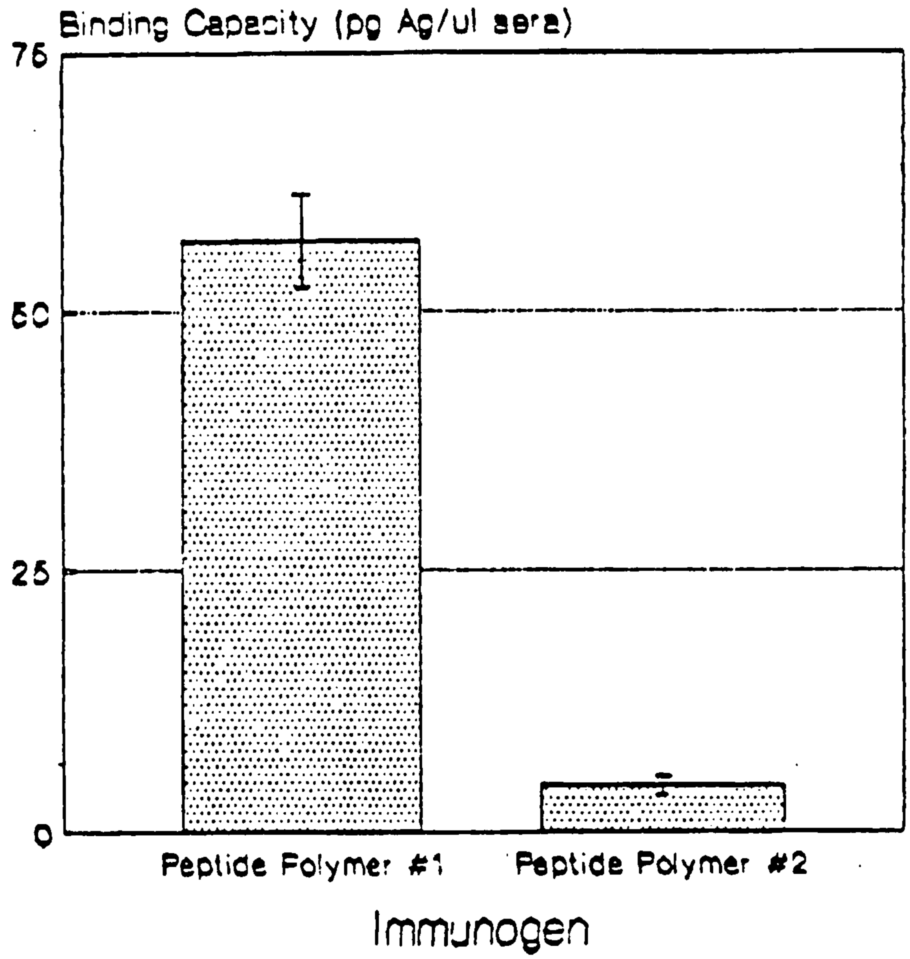
Stomach Acid Output in Response to
Injection of G17 and G34
in Control Rat



Arrows: Time of G17 or G34 injection.
Acid Secretion peaks are numbered 1,2,3.

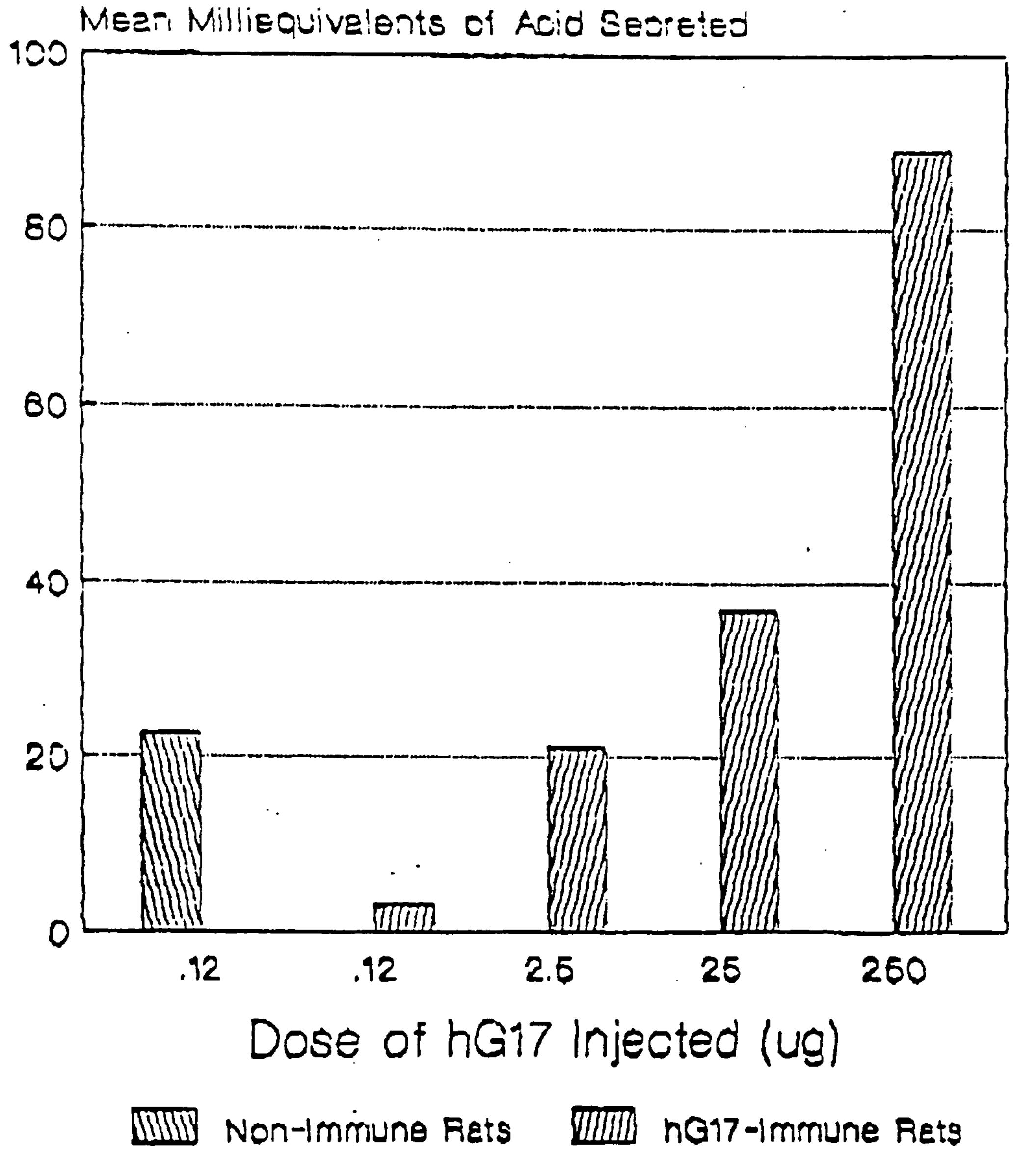
Figure 8

Mouse Antibody Responses to G-17 After Immunization with Polymerized Synthetic Peptides



Binding capacities determined by RIA, using (125-I)G-17. Values Plotted = Mean (- Bkgnd) and s.e; n = 5.

Figure 9 Total Quantity of Acid Secreted by hG17-Immune and Non-Immune Rats in Response to Graded Doses of hG17



n=6 for rats injected with .12 & 2.5ug.
n=2 for rats injected with 25 and 250ug.

FIGURE 10

Stimulation of the Growth of Human Colon Cancer Cell Line HCT 116 By Pentagastrin

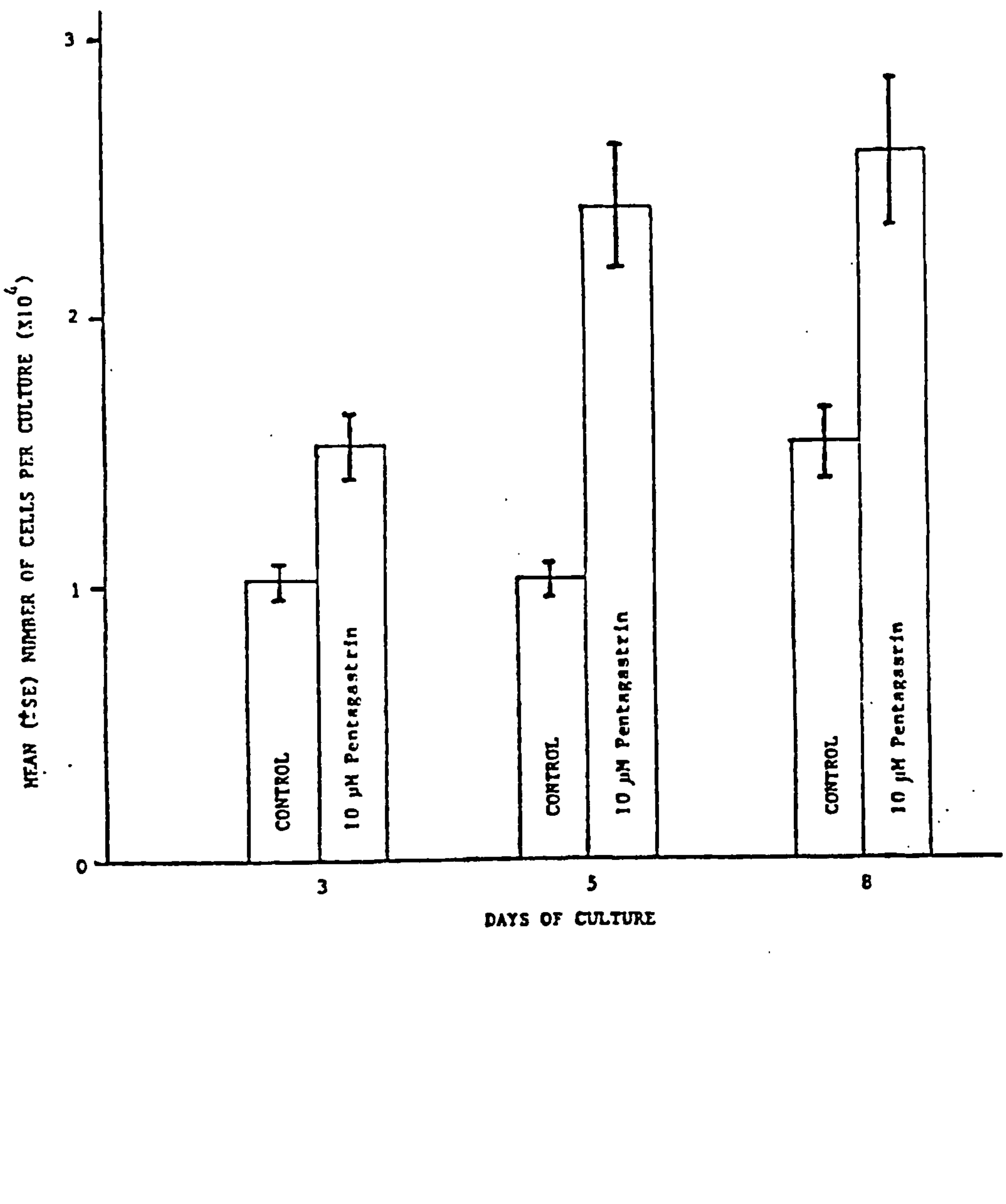
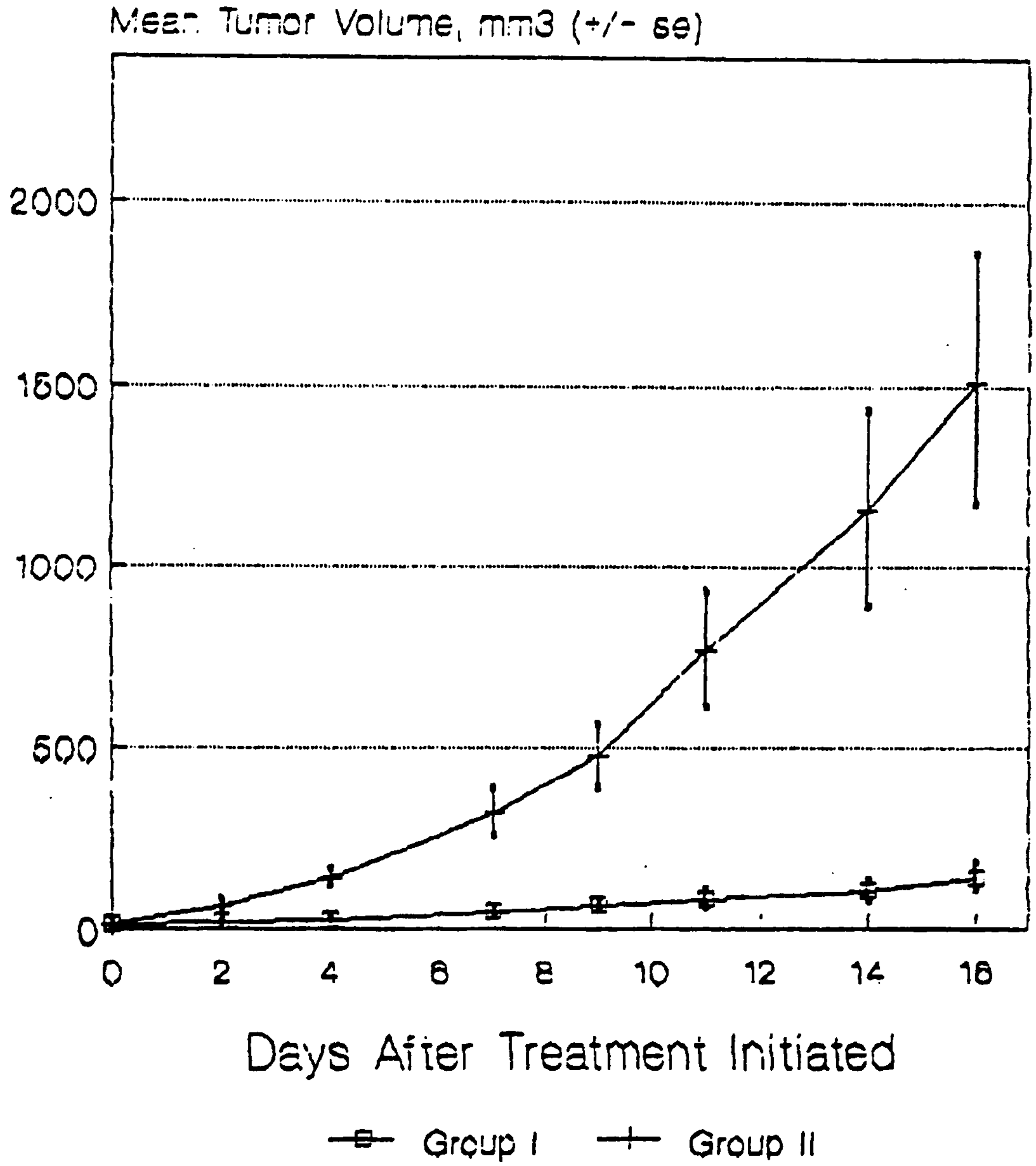
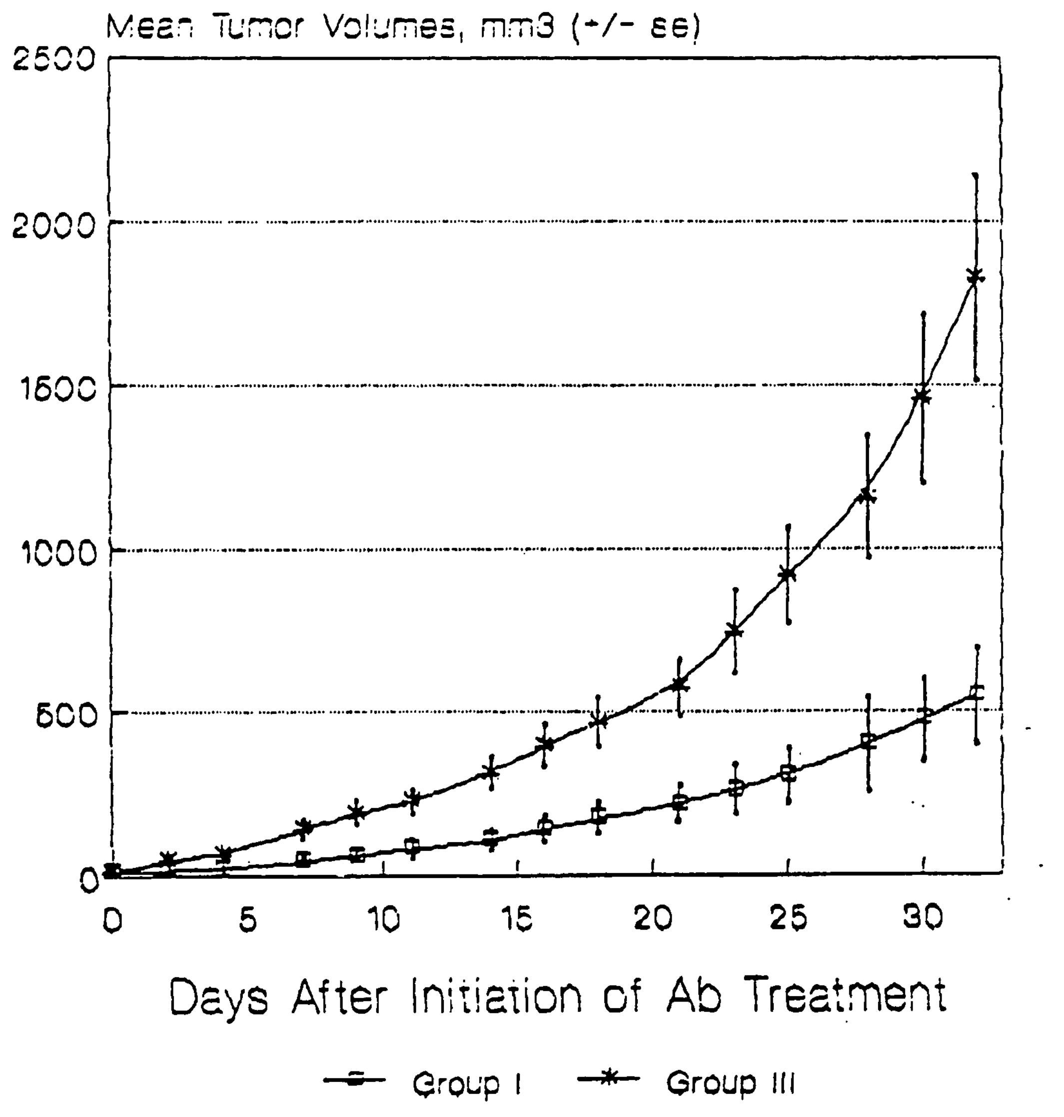


Figure 11 : Effect of Anti-G17 Ab on the Growth of Colon Cancer Implants in Nude Mice Infused with G17



Gp I: Injected with Rat Anti-G17 Ab.
Gp II: Injected with Normal Rat Ab.

Figure 12 : Effect of Anti-G17 Ab on the Growth of Colon Cancer Implants in Nude Mice



Group I: Injected with G17 and Anti-G17.
Group III: Injected with saline (no G17) and not injected with Ab.